JOURNAL OF AGRICULTURAL RESEARCH

VOL. 35 WASHINGTON, D. C., DECEMBER 1, 1927 No. 11

TYLENCHUS PRATENSIS AND VARIOUS OTHER NEMAS ATTACKING PLANTS[1]

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SYNONYMS AND ECONOMIC SIGNIFICANCE OF TYLENCHUS PRATENSIS

A large number of specimens from roots of various hosts were at hand for the study of *Tylenchus pratensis*.[2] This organism was described by De Man in 1884 as a rare species living free in the soil and at that time represented as having a definite posterior esophageal bulb, a statement which greatly influenced the further development of knowledge of this form (*6*, tab. xxii, fig. 95).[3]

In 1917 Cobb (*3*) described *Tylenchus penetrans* as a root parasite of potatoes, violets, cotton, and the camphor tree, and in 1919 (*4*) he emphasized the close relationship of *T. pratensis*, *T. penetrans*, and *T. musicola*. In a later note in his files he came to the conclusion that *T. pratensis* and *T. penetrans* were identical. The material which led him to this view was lily-of-the-valley roots infested with *T. penetrans* from Wageningen, the Netherlands, which came, therefore, from the same country from which De Man had obtained his original material.

In 1924 *Aphelenchus neglectus* was described by Rensch (*13*) from various host plants in Germany. This species in the conception of the writer is identical with the material under consideration, which he consideres beyond doubt to be *Tylenchus penetrans* of Cobb = *T. pratensis* of De Man.

In summarizing previous and present observations, *Tylenchus pratensis* must be regarded as an important widespread plant-parasitic nema of a pronouncedly destructive nature. It seems, therefore, that a clear definition of this species is of much importance.

Of all the descriptions given, that of Cobb is undoubtedly the most complete and accurate, and the specimens used in this study could easily be identified as his form. What apparently led him to create a new species under the name of *Tylenchus penetrans* was the very definite posterior esophageal bulb which De Man, one of the keenest of observers, draws in his figure of *T. pratensis*, together with the classification of the latter as a free-living form. However, Cobb, as well as the writer, has seen specimens of *T. penetrans* which

[1] Received for publication Aug. 15, 1927; issued January, 1928.

[2] For a part of the material dealt with in this paper the writer is indebted to E. G. Arzberger, of the Office of Nematology, who kindly called attention to several forms of nemas found during examinations of roots of a number of crop plants for their endophytic fungi. The grape roots infested with *Tylenchus musicola* were sent in by C. E. Scott, State of California Department of Agriculture, Sacramento, Calif. The lily-of-the-valley pips mentioned in connection with *Tylenchus pratensis*, the freesias infested with the root-knot nematode, as well as the cattleya plant mentioned later, were brought by Freeman Weiss, of the Office of Vegetable and Forage Diseases.

[3] Reference is made by number (italic) to "Literature cited," p. 981.

might account for De Man's drawings. In such specimens the glands and the tissue just in front of the intestine form a complex which could be conceived of as a posterior bulb. Although *T. penetrans* of Cobb, *Aphelenchus neglectus* of Rensch, and the writer's forms agree with *T. pratensis* of De Man in all other essential characters, they show this posterior esophageal bulb to be very indistinct. It can therefore be understood that Rensch, who had no males to examine and was looking for a well-developed posterior bulb, came to the conclusion that his specimen did not belong to Tylenchus but to Aphelenchus. However, if he had had more experience with free-living nemas, he would have noticed that the whole appearance and character of his specimens were more like those of Tylenchus than of Aphelenchus. There is, for example, the outlet of the salivary glands emptying dorsad into the esophageal tube just behind the spear—a feature not yet observed in any Aphelenchus. The figure Rensch gives is very rough and incomplete; the anal opening is not shown, nor the nerve ring, excretory pore, renette, or salivary glands. But all the facts Rensch gives about the size, morphology, and ecology of his animals agree so well with the writer's specimens that he considers them undoubtedly the same species. The writer, too, had not seen males until he had some fresh lily-of-the-valley pips, in which of 101 infesting specimens examined, 50 per cent were larval, 36 per cent females, and 14 per cent males, the sex number being 38.8. De Man mentions having had only one male; in Cobb's material, too, the females far outnumbered the males. This leads to the conclusion that they are either rare or have such different life habits from the females that they more easily escape observation. From a closer examination of conditions in lily-of-the-valley roots, it seems that the adult males more often stay on the surface of the roots or in the surface layers of the tissue. This points to the possibility that copulation takes place outside the root tissue.

Perhaps Rensch was unable to locate males, in spite of apparently plentiful material and after examining the surrounding soil, because he was looking for an Aphelenchus male to go with his supposed Aphelenchus females.

The fact that De Man's specimens were found living free can hardly be made a point against the identity of his and the writer's form. He had only three specimens and considered the form extremely rare; this corroborates the writer's observations of soil about the roots of Sisyrinchium, from which he was able to obtain only a few specimens, whereas within the roots they were fairly abundant.

A further point in favor of considering *Tylenchus penetrans* identical with *T. pratensis* is a note in Cobb's files referring to a cablegram from the Netherlands stating that *T. pratensis* was common there in roots of lily of the valley at the time Cobb examined roots of the same plant from Wageningen submitted by the Federal Horticultural Board. These lily-of-the-valley roots were infested with what he considered *T. penetrans*. The writer has observed the form in large numbers of lily-of-the-valley roots imported from Germany, where Rensch considered this *Aphelenchus neglectus* such a common pest. (Fig. 1.)

The situation to-day, therefore, is such that *Tylenchus pratensis* De Man 1884 must be considered as synonymous with *T. penetrans* Cobb 1917 and *Aphelenchus neglectus* Rensch 1924. The morphology of the form has best been worked out by Cobb. The writer has little to add to his description.

A study of living specimens revealed in an interesting way the ever-changing aspect which the annulation of the cuticle presents—dense with the contracted body and wide with the expanded. As Cobb remarks, the annulation continues even to the head end, which is set off in a buttonlike manner. (Fig. 2, A.) As many as three annules could be counted on the lip region. The cuticularized skeleton of this region is entirely interior and represents a very complicated framework. (Fig. 2, E.) The periphery of each lip is supported by an arcuate apophysis. This connects with a framework that surrounds the vestibulum and forms both a base to the lip region and a tubular structure backward, apparently some sort of guiding ring for the spear. Observing living specimens, it is noticed that the lip region is continually used as a sucking apparatus and in this case fixed on either the slide or the cover glass. Undoubtedly the whole framework is a means to develop these sucking qualities.

FIG. 1.—Portion of a lily-of-the-valley root infested with *Tylenchus pratensis* De Man. × 60

Micoletzky proposed in 1922 (*10, p. 542*) to place all the Tylenchi which have a cuticularized framework in the lip region in a separate subgenus, Chitinotylenchus. In 1925 (*11, p. 255*), however, he declared his subgenus Chitinotylenchus to be synonymous with Cobb's genus Tylenchorhynchus (*2, p. 438*). The latter genus, however, in its type species has two ovaries—a character which is not shared by all the members of Chitinotylenchus. As a generic or subgeneric character, this is considered more valuable than the cuticularized framework in the lip region. Therefore, it is perhaps better to regard Tylenchorhynchus distinct from Chitinotylenchus.

The writer was able to locate the amphids, although they are extremely small (Fig. 2, B.) They open on the exterior half of the lateral lip with an oval aperture. From here inward there leads a fine tubular canal, with thickened walls, ending just behind the lip region. Then follows a thin-walled section which resembles an extremely slender, elongated bottle. It could be followed back to a

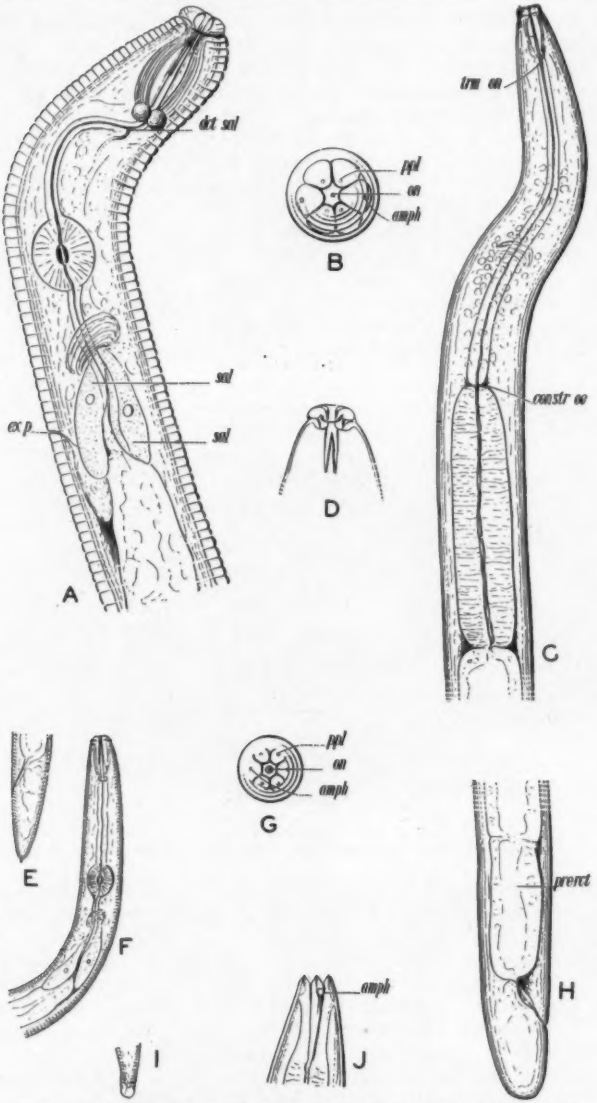

FIG. 2.—A, Anterior end of *Tylenchus tratensis* De Man; *dct sal*, outlet of the salivary glands; *ex p*, excretory pore; *sal*, salivary glands. × about 1,400.
B, Front view of head of same species; *amph*, amphid; *on*, spear; *ppl*, head papillæ. × about 2,100.
D, Molt of the head end of same species. × about 1,400.
F, Anterior end of *Tylenchus musicola* Cobb. × about 500.
G, Front view of head of same species; *amph*, amphid; *on*, spear; *ppl*, head papillæ. × about 2,100.
E, Tail end of a larval specimen of same species. × about 500.
I, Tail end of an adult female of same species. × about 500.
J, Head end of *Cephalobus oxyuroides* De Man; *amph*, amphid. × about 1,400.
C, Anterior end of *Axonchium parvum* n. sp.; *constr oes*, constriction of the esophagus (typical for this genus); *trm on*, end of spear. × about 700.
H, Tail end of same species; *prerect*, prerectum. × about 700.

short distance behind the base of the spear. A number of fibers (terminals) were seen inside. The whole organ is extremely fine and was best seen in specimens in exitu.

Head papillae were not seen. Even in front views it was not possible to ascertain their presence.

Figure 2, D, shows the molted head cuticle, which is of some interest because all the framework of the lip region is contained in this molt. As Cobb has described it from other Tylenchi, the spear is molted only in its anterior conical part, which is distinctly set off from the cylindrical posterior part. Cobb's conception of the difference in origin of the anterior part of the spear and the posterior part seems therefore well founded.

It was noticed that the lateral wings, too, were annulated, but that here one annule was as wide as two of the rest of the cuticle; the space between the wings also shows longitudinal striae, at least one on each side. In one specimen a much larger number was seen in the vulvar region.

As has been remarked, De Man found his few specimens free in the soil. Cobb (*3*) in his paper on *Tylenchus penetrans* gives four important hosts, and in his unpublished files two others, the lily of the valley and the fig tree. Rensch mentions a number of hosts from Germany and notes their pathological features. They consist of restricted growth and yellowing of the leaves—features which most often are attributed to other causes than nema infestations. This may be one reason why *T. pratensis* as a plant parasite has hitherto been given so little attention by plant pathologists, although it appears to be a very widespread and serious nemic pest. The host list already includes the following plants:

Cobb's observations:
- Potato tubers (Michigan, Tennessee).
- Violet roots (New York).
- Cotton roots (South Carolina, Georgia).
- Camphor-tree roots (Florida).
- Lily-of-the-valley roots (Wageningen, the Netherlands). Unpublished.
- *Ficus carica*, fig-tree roots (California).

Rensch's observations:
- Winter rye (Germany).
- Wheat (Germany).
- Winter barley (Germany).
- *Weingartneria canescens* (Germany).
- Papaver plants (Germany).
- Wild oats (Germany).
- Atriplex plants (Germany).
- Sugar beets (Germany).
- Rape (Germany).

Writer's observations:
- Wheat (Arlington Experiment Farm, Rosslyn, Va., and Ohio Agricultural Experiment Station).
- Rye (Arlington Experiment Farm, Rosslyn, Va.).
- Corn (*Zea mays*) (near Chain Bridge, Va.).
- Sugar cane (greenhouse, Arlington Experiment Farm, Rosslyn, Va.).
- Cotton (South Carolina).
- Poplar hybrids (propagation plot of New York Botanical Garden).
- *Sisyrinchium angustifolium* (Woods Hole, Mass.)
- Lily of the valley (*Convallaria majalis;* Germany).
- Cattleya sp. (greenhouse near New York City).

The measurements given by the various authors are as follows:

De Man:

2.7	?	22.6	–79–	96.4	0.6 mm.
2.4	?	3.6	3.6	2	
3.3	?	20	M	95	0.45 mm.
	?	?	3.6	2.2	

Cobb:

2.8	12	18	80$^{78}_{5}$	95	0.7 mm.
3.1	4.2	4.5	4.6	2.6	
3.2	11	16	M	94.5	0.5 mm.
4	4.5	4.6	4.3	3.6	

Eggs.—Length, 0.078 mm.; width, 0.025 mm.

Rensch:

3.9	?	13	–75–	?	0.467 mm.
3.9	?	?	4.9	?	

Eggs.—Length, 0.063 to 0.068 mm.; width, 0.021 to 0.025 mm.

Measurements from the writer's specimens:

Average of three females—

2.9	12	17	80	95.6	0.58 mm.
2.5	3.4	3.5	3.7	2.5	

Eggs (average of 5).—Length, 0.067 mm.; width, 0.025 mm.

Average of three males—

3.2	15	20	M	95.8	0.5 mm.
2.2	3	3.2	3.2	2.4	

As pathological symptoms resulting from infestation by the present species, Cobb described peculiar pustules on potato tubers and brownish spots on the roots of violets. Such spots have been seen by the writer on roots of Sisyrinchium. In the lily of the valley, however, the spots, or sometimes whole areas, were reddish. With the exception of potato tubers, the nemas have been observed only in the cortex of roots, where they move in the parenchymal tissue and apparently feed on the cell contents. They force their way through the cell walls; often, especially in cross sections, one comes across a specimen which has its body "strangulated" by a cell wall which did not give way; but this apparently does not matter to the nema.

Very often one sees a large number of specimens quite close together, as shown in Figures 3, 4, and 5. Eggs, larvae, and adult females are seen together; as many as five eggs have been seen deposited tandem, separated by only a few plant cells. The eggs are large compared with the adults, but rather thin shelled. They develop inside the roots; the larvae hatch out blunt tailed and devoid of any signs of a spinneret. In agreement with the other observers, the writer has seen only a single egg at a time in the uterus.

Cobb has already emphasized the ecomomic significance of the present species. Rensch considers the present species very inju-

rious, especially to young plants and seedlings. He found the roots of seedlings only four weeks after they had been sown completely shot through with the nemas. But it is only necessary to look at Figures 1, 3, 4, and 5, which represent typical cases of the way these nemas occur in the finer roots, to realize that such a plant can not grow properly. The writer is convinced that the future will prove that in many instances troubles caused by the present form have wrongly been laid to some other cause.

Specimens of *Tylenchus pratensis* undoubtedly move slowly but continually through the root tissues. The path prepared by the larvae aids in the spread of other root diseases through the root tissues, especially fungi. Cases have been observed by the writer where numerous chytrids could be seen following what appeared to be the trail of *T. pratensis*.

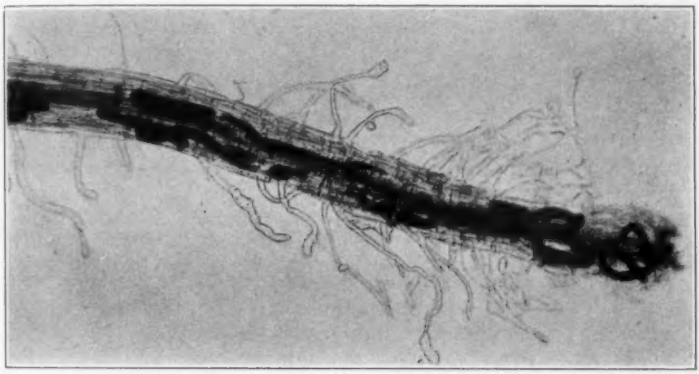

FIG. 3.—Root of wheat (Kanred) infested with *Tylenchus pratensis*. × about 55

HOPLOLAIMUS CORONATUS, A PARASITE IN ROOTS OF SUGAR CANE, CORN, RED CLOVER, AND ALFALFA

Hoplolaimus coronatus was only recently (1923) described by Cobb (*5*) from a few specimens found around a "nest of Mermis" near Falls Church, Va. The comparatively late discovery of this large and outstanding nema is probably due to its parasitic life, which keeps the specimens inside the roots of their host plants during most of their life period. The writer has frequently seen specimens leaving roots or just on the point of entering them. (Fig. 6.) This observation rather points to a vagrant mode of life. It is not known whether this species copulates inside or outside the roots, but its considerable size would indicate that copulation in the soil is more probable.

Hoplolaimus coronatus Cobb was first observed by the writer in roots of corn (*Zea mays*) from Virginia and was found to spread all through the fine roots; later it was noticed to be very common and abundant in roots of red clover and alfalfa from near the campus of the University of Maryland, College Park, Md.[4] In 1924 it was also found in roots of sugar cane grown in greenhouses at the Arlington

[4] For these samples the writer is indebted to H. S. McConnell, Division of Entomology, University of Maryland.

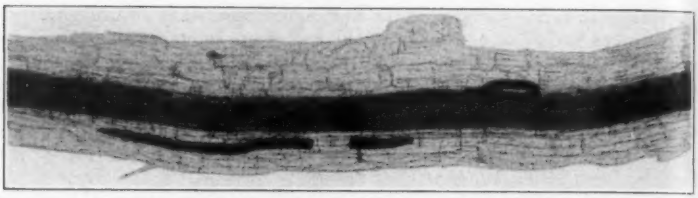

FIG. 4.—Root of rye infested with *Tylenchus pratensis*. × about 4 or 5

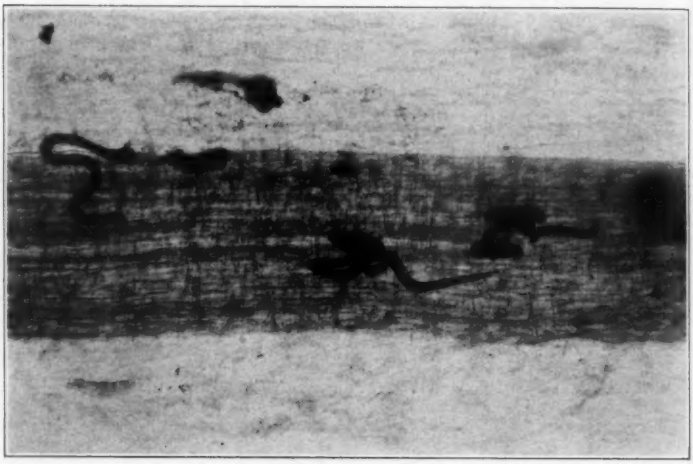

FIG. 5.—Root of a cotton plant infested with *Tylenchus pratensis*. × about 55

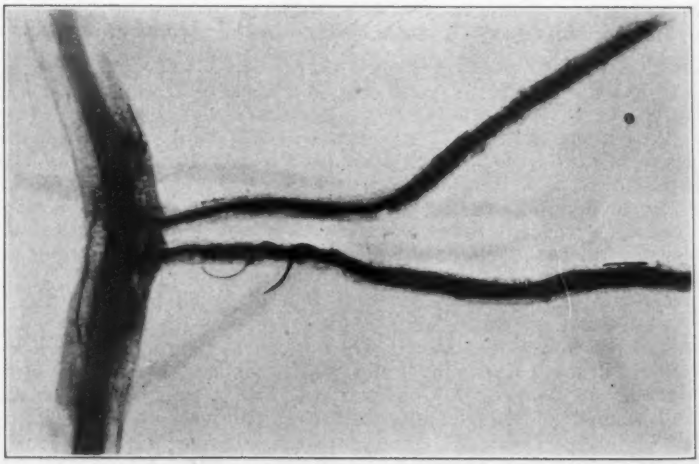

FIG. 6.—Corn roots with *Hoplolaimus coronatus* Cobb. Two specimens are entering the roots. × about 8

Experiment Farm, Rosslyn, Va. In a recent paper F. Muir mentions two Hoplolaimi from sugar-cane roots from Hawaii but does not name the species of Hoplolaimus. Within the roots, these nemas are always located in the parenchyma of the cortex, and the writer has never seen them elsewhere. Although a few specimens may occasionally be found together, this form never occurs in "nests," like *Tylenchus pratensis*.

Hoplolaimus coronatus Cobb is probably widespread, attacking a much larger number of plants than the few mentioned above. Its significance may not be limited to the destruction of the root tissues and interference with the physiological function of the roots. On account of its considerable size it may be an agent which prepares an entrance for other diseases and thus aids in spreading them.

TYLENCHUS MUSICOLA LIVING IN ROOTS OF GRAPE

It was reported by Cobb in 1918 that *Tylenchus musicola* Cobb from roots of the Bluggoe banana, Grenada, Barbados, West Indies, caused a serious disease. The present findings from roots of grapes (*Vitis vinifera*) from Loomis, Calif., are the second ones, thus showing that this nema is not confined to the banana and the Tropics.

The nemas were found all through the parenchymal tissue, close to the central cylinder; larvae and females were observed, but no males were found. The grape roots had swollen tips and were therefore examined for root knot (*Caconema radicicola*), which led to the finding of the infestation by the present form. Unfortunately, the roots as received were quite dry; hence the material was not of the very best. Cross sections proved that the cell walls were greatly damaged and apparently broken to a considerable extent by the moving nemas. Seemingly the cells are emptied of the plasmatic content.

The characters given by Cobb apply very well to the specimens received. (Fig. 2, D.) The writer is convinced that not only "phasmids" (lateral papillalike organs in the middle of the tail end in the male and female) but also "deirids" (lateral papillae in the cervical region) are present; the latter were observed in the latitude of the anterior esophageal bulb. Regarding the spear, Cobb writes: "In the preserved specimens the anterior portion of the spear is distinctly visible, but the bulbs and hilt are rather ghostly, though well developed." This characterizes in the most accurate way the conditions observed in the writer's specimens. The posterior esophageal bulb is very indistinct. The salivary glands were seen, but not their outlet behind the base of the spear. However, an ampullalike swelling of a glandular nature was observed inside the esophageal tissue. Cobb has already emphasized the variability of the shape of the tail end. In the writer's material the tail was usually bluntly rounded, as shown in Figure 2, I. In the larval specimens a small mucro was often seen (fig. 2, E); sometimes this mucro was shifted to the ventral side of the then rather blunt end.

In studying the front view of the head (fig. 2, G), four submedial but rather faint papillae were seen. The amphids are also very inconspicuous and somewhat closer to the periphery of the head. The measurements were found to be as follows:

$$\text{Female 1: } \frac{2.6}{1.9}\ \frac{12}{2.9}\ \frac{19}{3.2}\ \frac{79}{3.2}\ \frac{95.5}{2.3}\ 0.64 \text{ mm.}$$

Female 2:	3	14	21	79	93. 9	0.54 mm.
	2. 3	3	3. 4	3	2. 3	
Female 3:	3. 1	14	23	78	93. 8	0.52 mm.
	2. 3	3. 1	3. 5	3. 5	2. 7	

RELATIONSHIP OF SOME SO-CALLED SOIL NEMAS TO CORN SEEDLINGS

For the study of the relationship of some chytrids to corn roots, the following experiment was arranged:[5] A handful of soil was placed in the bottom of a cylindrical glass jar about 75 cm. in height. The soil was known to contain chytrids, but no special observations were made concerning its nemic fauna, although it was known that *Hoplolaimus coronatus* was present at the place where the soil was collected. After the soil was carefully placed in the bottom of the jar, the latter was cautiously filled with running water and a corn seed placed on top of the water column now about 66 cm. high. The seed itself had been treated with bichloride of mercury. After four weeks the seedling was removed and the roots subjected to a very careful examination. The search revealed a number of nematodes within the roots. From a piece of root about 2½ cm. long 58 specimens were isolated. The forms observed belonged to six different species: 1, *Dorylaimus brigdammensis* De Man; 1, *Axonchium parvum* n. sp.; 2, *Aphelenchus parietinus* Bastian; 18, *Cephalobus oxyuroides* De Man; 1, *Plectus granulosus* Bastian; 35, *Plectus longicaudatus* Bütschli.

These findings are of some significance because they throw a more definite light upon the relationship of the forms mentioned to plants, especially to corn. In phytopathological circles usually very little consideration is given to the so-called soil nemas. All the above-mentioned forms, however, belong to this group and have a much closer relationship to plant life than was once thought. The nemas were apparently able to locate the growing seedling, which at the beginning was at least 60 cm. distant. The attraction which the seedling exerted was probably based on chemical secretions which diffused through the water and served as a directing agent. To cross the distance the nemas had either to move in an almost perpendicular path through the water or to use the wall of the glass jar as a climbing support. But in either case what appears to be a developed sense of "smell" is rather outstanding. This point calls for even more consideration because the forms involved were not thought to have plant-attacking habits. It must be admitted that the soil used in this experiment was taken from a cornfield around the corn roots. The nemas had therefore probably been familiar with corn as a food plant and so may have responded more accurately to it than they would have responded to any other plant.

Of all the species present, *Plectus longicaudatus* Bütschli was most numerous; 12 adult females and 23 larvae of various sizes were observed; 7 females had eggs. Contrary to observations of earlier investigators (*7*, *10*), most of the specimens had distinct lips. This would bring them closer to *P. rhizophilus* De Man, or, as Micoletzky conceived it, to *P. cirratus* Bastian var. *rhizophilus* De Man; but the size, the shape of the tail, the structural conditions of the cardiac

[5] By E. G. Arzberger, Office of Nematology.

bulb, the mouth cavity, and the amphids rather speak for systematic position near *P. longicaudatus*. The writer can support the description of the amphids which De Man gave in 1921 (*8*); their external parts have a spiral form with only one turn rather than a circular form. From this part a duct can be followed leading backward as far as a short distance proximad to the base of the mouth cavity; the duct always starts at the open edge of the spiral, i. e., it is always dorsad. Another observation concerning the morphology of *P. longicaudatus* is the presence of a deirid, i. e., a fine lateral papilla on each side a short distance behind the nerve ring. Such papillae have not yet been mentioned as occurring in any of the members of the genus Plectus.

The number of specimens of *Plectus longicaudatus* present in the roots of corn seedlings proves beyond any doubt its ability to feed entirely on corn roots and to live and propagate within the root tissue. At least some of the younger larvae seem to have developed from eggs which the adult forms had deposited within the roots. *P. longicaudatus* is syngonic, and therefore propagation is not dependent upon the presence of males. This may account for the fact that *P. longicaudatus* was more numerous in the present case than *Cephalobus oxyuroides*. A careful study of the intestinal content of the specimens was made, but only finely granulated materials were seen. It is therefore concluded that *P. longicaudatus* fed directly on the cell contents of the root parenchyma in which it was found. Although the water roots of the present seedling represented especially soft tissue, this alone would not account for the presence of all the nemas in it. There is certainly a definite food relationship between the nemas studied and the corn plant. The measurements of two adult specimens of *P. longicaudatus* were as follows:

0.54	15	27	'51'	85	0.38 mm.
1.3	3.2	4.3	5	2.2	
0.66	13	25	50	86	0.43 mm.
1.2	3.3	4.1	4.7	1.9	

These measurements agree well with those given by earlier observers—a fact which indicates normal living conditions in the present case.

Cephalobus oxyuroides De Man was next in number to *Plectus longicaudatus*. It is a species which had been observed by the writer very frequently in various tissues of numerous plants. This form also seems able to live upon plants entirely. The corn roots studied contained 7 adults (4 males and 3 females) as well as 11 larval forms, and it is thought that copulation and the deposition of eggs occurred within the root parenchyma.

The head end of the present species, shown in Figure 2, H, J, may aid in completing our knowledge of the head sense organs. As can be seen, the six lips have two fine papillae each; the amphids are situated on the dorsal part of the lateral lip, close behind the lateral papillae. This species too has phasmids (lateral papillalike organs in the middle of the tail in males as well as females) and deirids.

The writer has already called attention to the economic significance of various other members of the genus Cephalobus in other papers. The present observations afford additional evidence. Many Cepha-

lobi, the present species included, may be omniphagous, at least to a certain degree. Although this character makes less significant the extent of direct damage done by this species of nemas, on the other hand it causes them to serve as important carriers for diseases (bacterial, virus(?), and fungous).

Dorylaimus brigdammensis De Man was found, but only a single specimen. It had not yet been recorded from America, but was known to live in the soils of the Netherlands, Switzerland, Denmark, and Russia. Its food and feeding habits were not known. The present observation suggests a parasitic relationship to plants. The specimen had an egg in its uterus. The measurements were as follows:

$$\frac{0.14 \quad 7.4 \quad 22 \quad -48- \quad 83}{0.69 \quad 1.8 \quad 2.2 \quad 2.2 \quad 1.5} \text{ 1.5 mm.}$$

Axonchium parvum n. sp. is the smallest of all known members of this genus. Although some Axonchia had been found around the roots of plants, none had been observed in plant tissue.[6] The present specimen was an adult female, but had no egg in the uterus, although one was ready to enter it. Doubtless this form can live entirely parasitically.

The distinguishing characters of the new species are as follows:

$$\textit{Measurements.}— \frac{0.64 \quad 8.7 \quad 27 \quad 51— \quad 97.4}{1.1 \quad 2.9 \quad 3.5 \quad 3.7 \quad 2.2} \text{ 0.64 mm.}$$

Diagnosis.—Axonchium of small size (0.64 mm.); the enlarged posterior part of the esophagus less than one-half its length; tail end bluntly rounded, ovary posterior, with the end reflexed and reaching two-thirds back to the vulva; male unknown.

Only a few species of this well-defined genus [7] are described. The present one is much smaller than all the others and especially well characterized by the short posterior part of the esophagus. It is very well set off by the deep constriction so typical of the genus. The writer was unable to determine definitely the number of head papillae, but estimates that there are two circle of six each, the lips being only faintly visible. The spear is about three times as long as the head is wide and, at least in the present specimen, rather fine. (Fig. 2, C and H.)

Plectus granulosus Bastian was represented in the corn roots by a single adult female with the following measurements:

$$\frac{2.2 \quad ? \quad 21 \quad {}^{14}52^{15} \quad 92.3}{1.8 \quad ? \quad 3.3 \quad 3.6 \quad 2.2} \text{ 1.02 mm.}$$

This form has already been claimed by several observers as feeding, at least partly, in plants. It has been mentioned from seedlings of cereals, and Micoletzky (*10*) found chlorophyll as intestinal content.

Aphelenchus parietinus Bastian was represented by an adult female with one egg and a very young larval specimen. The measurements of the female were as follows:

$$\frac{1.8 \quad 4.9 \quad 12 \quad \underline{38}\,67 \quad 91.3}{1.8 \quad 2.3 \quad 2.7 \quad 2.9 \quad 1.5} \text{ 0.42 mm.}$$

[6] Since this was written F. Muir states that Axonchium was living in sugar-cane roots in Hawaii (*12*), but he does not mention the species.

[7] It is a great mistake to consider this well-characterized genus as synonymous with Dorylaimus, as do Baylis and Daubney in a recently published book (*1*).

Following Micoletzky's conception of this species, the present form would come under the variety *tubifer*, forma *parvus*, subforma *informis*. *A. parietinus* has been recorded from various hosts by several investigators.

UNUSUAL REACTIONS OF FREESIAS TOWARD THE ROOT-KNOT NEMATODE (CACONEMA RADICICOLA)

A conception commonly found among plant pathologists is that nemic diseases of plants can be recognized with much accuracy by certain pathological symptoms exhibited by the host plants. This is often the case, but appearances are also often misleading.

Plants with nemic infestation might sometimes (perhaps under certain climatic or growth conditions) show no symptoms, and in other cases symptoms regarded as typical for nemic diseases might exist and no nemas be found. Nematologists therefore are rather skeptical about such symptoms and consider an examination with the microscope the only safe way to determine the presence of nemas. Besides, as already shown in the present paper, a number of injurious plant-parasitic nemic species never produce special symptoms, i. e., symptoms which could not be attributed also to some other cause.

Even such a nemic disease as root knot, seemingly well character ized by its symptoms, is not always properly recognizable by them. Peonies, for example, have been observed in this laboratory in numerous instances to exhibit root swellings with all the typical features of root knot, and yet *Caconema radicicola* could not be located. On the other hand, roots of plants, e. g., cyclamen, have also been observed which, although they showed no swellings harbored the root-knot nema and were severely affected. It is evident that various plants do not react in the same way toward the root-knot nema. Even early observers noted that some heavily infested plants seemed to suffer little or not at all, whereas other species with comparatively few knots were in a dying condition. Cyclamen is in this respect an outstanding example. It was observed that a few specimens of *Caconema radicicola* were able to kill half-grown plants. The roots did not develop the typical knots, but began to decay at the places where the parasite was located; in some such instances the nemas could be located only after search somewhat more tedious than usual, because the absence of swellings left the observer without the usual characteristic guide.

Recently the writer came into possession of some freesia bulbs sent in for examination by a New Jersey nursery because of their failure to bloom after forcing. The bulbs were infested with *Caconema radicicola*. This plant may be added therefore to about 660 other hosts already known. Most interesting, however, were the symptoms of this infestation. Contrary to the usual development where the whole root swells at the location of the parasite, such swellings were absent. In the fully developed stage brownish-yellow spherical bodies are attached to the roots. (Fig. 7.) They stand out especially well on the finer rootlets. At first sight they resemble the brown cysts of the sugar-beet nematode (*Heterodera schachtii*) and could be mistaken for such by inexperienced observers. However, a close examination of the adult female in these globules as well as of the larvae proves at once that it is the typical root-knot nema.

A comparison of such nodules at different stages of development (figs. 7 to 9) leads to an understanding of these anomalous conditions. In plants with a normal reaction toward the nema and the young female Caconema develops within the parenchyma of the root cortex. As the female begins to grow and swell, it presses toward the surrounding tissue, which naturally slackens on the side of least resistance, that is, toward the surface. Here a more and more pronounced elevation is formed. The tissue of freesia, however, seems to be unable to counteract or neutralize this expansion by regenerative action and simply cracks and splits, the outer tissue assuming a brownish discoloration. Eventually it dies off and forms the above-mentioned yellow-brown crust around the female nema,

FIG. 7.—Roots of freesias with the females of *Caconema radicicola* attached outside the roots × 4½

FIG. 8.—Roots of freesias with immature females of *Caconema radicicola*. × 4½

which appears more or less outside the surface of the root and at least partially free. These conditions are understood to be a result of the lack of regenerative power in the root cortex tissue of freesias. Whether this is a general lack or a specific absence resulting from the influence of the nema, the writer is unable to judge. Freesia, however, evidently suffers much from a few Caconema parasites and belongs to that group of plants easily damaged by even a small infestation. It seems from the foregoing description that the symptoms are more nearly those of *Heterodera schachtii* than those regarded as typical for *Caconema radicicola*, proving once more that too much stress should not be laid upon the macroscopic pathological symptoms in diagnosing nemic diseases.

REMARKABLE HABITAT OF TYLENCHUS DIPSACI

The stems, leaves, flowers, and seeds as well as certain subterranean stems and leaves (bulbs, tubers, and rhizomes) are the parts of plants usually chosen by *Tylenchus dipsaci* to live in. The recognition of bulbs, tubers, and rhizomes as types of stems and leaves proves much for the "botanical" instinct of this nema. So far as the writer knows, *T. dipsaci* has never heretofore been recorded from the roots of plants. The recent discovery of this nema in the roots of lilac

FIG. 9.—A single female of *Caconema radicicola* just breaking through the root surface of a freesia. × about 60

hybrids (*Syringa vulgaris*) therefore came as a surprise. These plants were imported from Nancy, France, and were submitted for examination because of warty outgrowths on the roots (crown galls). Nemas were also found, and these proved to be *T. dipsaci*. Although their number was not large, several were obtained, mostly larvae. At least a part of the specimens came from the above-mentioned warts.

The measurements of an adult male were as follows:

1.2	9.1	17	M	90.9	0.7 mm.
1.4	2.5	2.8	3.3	2	

NEMAS ASSOCIATED WITH THE ROOTS OF AN ORCHID

A cattleya plant sent in for examination from a greenhouse near New York City afforded the opportunity to investigate, in a preliminary way, the nemic fauna associated with its roots. The cattleya, especially its root system and the soil in which it grows, undoubtedly represents highly specialized conditions for the life environment of a nemic fauna. It was therefore expected that a number of widely different forms, able to live under the most varied conditions, would be found. On the other hand, it was thought that perhaps some forms particularly adapted to the specialized conditions here existing might be observed. These expectations were realized. As may be seen from the list here given, most of the nemas isolated are forms able to live under most varied conditions. But there are also two forms which perhaps are peculiar to such orchids, namely, *Aphelenchus tenuicaudatus* De Man and *Diplogaster asymmetricus* n. sp. The former was described by De Man from the roots of orchids, namely, various species and hybrids of calanthes, and has never been found since, as far as the writer knows. *Diplogaster asymmetricus* is a new species and was the most numerous species present, a fact which seems to indicate that it is perhaps a form specific for orchids.

The nemas listed below all came from the surface or from the inside of the roots, only parts of which were examined. Their close connection with these roots is beyond doubt, although the exact nature of this relationship is not known. But that these relations might often be of indirect and complex character is to be concluded from the fact that hyphae of a mycelium were observed in the intestine of a *Plectus communis* found on these cattleya roots. This suggests that this nemic species is associated with the mycorrhiza of this orchid—a new viewpoint for the ecological significance of the nemic fauna of soils.

The nemas isolated from the roots of the cattleya plant included 100 specimens belonging to 15 different species and to 10 different generas, as follows: 14, *Rhabditis monhystera* Bütschli; 2, *Plectus longicaudatus* Bütschli; 1, *Plectus communis* Bütschli; 21, *Cephalobus oxyuroides* De Man; 5, *Cephalobus longicaudatus* Bütchli; 6, *Acrobeles* (*Acrobeloides*) *minor* Thorne;[8] 2, *Acrobeles tricornis* Thorne;[8] 26, *Diplogaster asymmetricus* n. sp.; 1, *Prismatolaimus intermedius* De Man; 1, *Bastiania longicaudata* De Man; 3, *Monhystera vulgaris* De Man; 3, *Tylenchus pratensis* De Man; 1, *Tylenchus intermedius* De Man; 13, *Aphelenchus tenuicaudatus* De Man; 1, *Aphelenchus* sp.

Thus the nemic component of the root biocoenosis of cattleya proves itself to be rich in numbers as well as in forms.

RHABDITIS MONHYSTERA

A number of larval and female specimens of *Rhabditis monhystera* Bütschli were seen. De Man (*9*) has recently called attention to the fact that in the past at least three different species were wrongly placed under this name. The writer's specimens, however, surely belong *to* Bütschli's form.

[8] For the identification of these species the writer is indebted to Gerald Thorne, of the Office of Nematology.

PLECTUS COMMUNIS

Only a single specimen of *Plectus communis* Bütschli was found—an adult female. Deiride were present; possibly also phasmids. The amphidial openings are not transverse oval openings as would be expected from De Man's drawings, but they have the shape of a defective spiral, open dorsoposteriorly. This form seems to live, at least partly, on fungus mycelium. A partly digested mycelial thread was distinctly seen in the intestine.

CEPHALOBUS OXYUROIDES

Some specimens of the present material are considered to be *Cephalobus oxyuroides* De Man, although they are of very dwarfed size—such as not yet mentioned in literature. Of three males seen and measured, the smallest was only 0.38 mm. and the largest 0.41 mm.; yet, as may be seen from the following formulae, the size relations agree fairly well with what is known:

Male 1:	2.6 / 2.2	22 / 3.2	30 / 3.8	M / 4.3	91.4 / 3.2	0.38 mm.
Male 2:	2.4 / 2.2	21 / 4.1	30 / 4.1	M / 4.9	90.3 / 3.2	0.41 mm.
Female (fertilized, but without egg):	1.1 / 1.2	18 / 3.1	30 / 3.3	[24] 66 [21] / 3.4	91 / 1.9	0.42 mm.

The three papillae on the male tail end just in front of the terminal point could always be detected easily; this was not true of the other papillae, which were very faint and sometimes rather doubtful.

DIPLOGASTER ASYMMETRICUS

A considerable number of female, male, and larval specimens of the new species, *Diplogaster asymmetricus* n. sp., were seen. (Fig. 10, A, C, D, E.) Its name was chosen because of the asymmetrical position of the pharyngeal teeth.

Measurements—

Female:	0.93 / 1.2	9.3 / 1.9	14 / 2.3	[17] 41 [18] / 3.2	74 / 1.8	0.88 mm.
Male:	0.75 / 1.2	9 / 1.9	14 / 2	M / 2.3	71 / 1.8	0.82 mm.

Diagnosis.—Diplogaster with a very elongated setaceous tail end (fig. 10, E) in both sexes; a mouth cavity wider than deep and about half as deep as the head is wide; only two pharyngeal teeth, one somewhat larger dorsally, slightly to the left and a submedial smaller one also to the left (fig. 10, C); middle esophageal bulb well developed (fig. 10, D); cardiac bulb indistinct, in fact only a gradual swelling of the posterior part of the esophagus; male with curved spicula and a somewhat flag-shaped gubernaculum, the staff of which points along the spicula to the anal opening, whereas the flag is directed caudad and has a rounded edge; a ventro-submedial setaceous papilla is in front of the inner end of the spicula, a second one in front of the anus, a third one back of the anus, and a group of three in about the middle of the conical, not filiform, part of the tail; there are also three lateral papillae, one in front of the anus, one close to the middle of the conical part of the tail and one about where the filiform tail end begins (fig. 10, E).

Description and remarks.—The tail end of this species is extremely fine, but breaks off apparently quite easily. It is not difficult to recognize the annulation

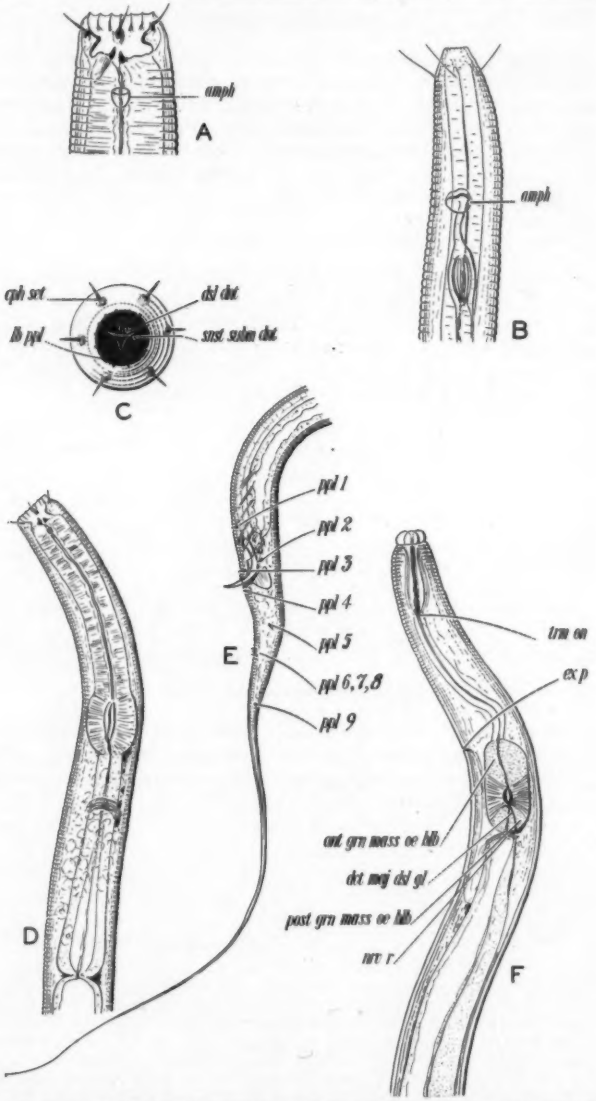

FIG. 10.—A, Head end of *Diplogaster asymmetricus* s. sp.; *amph*, amphid. × about 1,400.
B, Head end of *Bastiania longicaudata* De Man; *amph*, amphid. × about 1,400.
C, Front view of head of same species; *cph set*, cephalic setae; *dsl dnt*, subdorsal onchium; *snst subm dnt*, left ventro-submedial onchium; *lb ppl*, labial papillae. × about 1,400.
D, Anterior end of same species. × about 500.
E, Tail end of male, same species; *ppl* 1 to 9, copulatory papillae. × about 500.
F, Anterior end of *Aphelenchus tenuicaudatus* De Man; *ant grn mass oe blb*, anterior granular mass of the middle bulb of the esophagus; *dct maj dsl gl*, ductus of the large dorsal gland; *ex p*, excretory pore; *nrv r*, nerve ring; *post grn mass oes blb*, posterior granular mass of the middle bulb of the esophagus; *trm on*, posterior end of spear. × about 500.

of the cuticle, but it is hard to distinguish the elements of this annulation. These are transverse series of points which seemingly are arranged also in longitudinal series at the conical part of the tail end. The oral opening is wide and surrounded by a membranelike structure which is supported by 14 labial papillae. (Fig. 2, A and B.) These give the walls of the oral cavity a striated appearance when seen from the side. There are six single cephalic setae, each placed on a thickened base. The amphids are located a short distance back of the mouth cavity; the opening is a transverse oval and leads into a cuplike cavity.

On the base of the wide mouth cavity are the two rather small onchia, slightly curved and pointed forward; the side walls of the oral cavity are strong and supported by mobile cuticularized pieces. (Fig. 2, A.)

The cylindrical anterior part of the esophagus back to the middle bulb has radial muscles very well developed, whereas the posterior part consists of an entirely different kind of tissue and gradually swells to form a bulklike end. It seems that this posterior part of the esophagus contains some glandular cells, one of which stands out prominently because of its remarkable nucleus. No excretory pore and no renette cell were seen.

The female sexual organs are double, the ovaries reflexed, and there is in each uterus usually only one egg, which apparently is deposited in an advanced stage of segmentation.

The most remarkable character of the male is its gubernaculum, which, seen from the side, somewhat resembles a flag with a rounded contour and with a staff handle turned toward the anus. Of all the Diplogasters with similar gubernacula, the one of the present species is the largest the writer has ever seen. There are indications that the protrusor muscle of the spicula is attached to the sides of this gubernaculum. A few oblique copulatory muscles were seen in front of the anus.

PRISMATOLAIMUS INTERMEDIUS

The only specimen of *Prismatolaimus intermedius* De Man was a female of dwarf size, reaching only 0.045 mm., thus remaining considerably below the minimal size given by various observers. The vulva opened at 62 per cent of the total length. A careful examination of the head end proved that Micoletzky is right in attributing to this species 10 instead of only 6 cephalic setae, confirming also his description and figure of the amphids.

BASTIANIA LONGICAUDATA

A single female specimen of *Bastiania longicaudata* De Man (fig. 10, B) was found, with measurements as follows:

$$\frac{0.19 \quad 6.2 \quad 18 \quad {}^{14}57^{15} \quad 90}{0.58 \quad 1.0 \quad 1.2 \quad 1.9 \quad 0.97} \text{ 1.06 mm.}$$

Since the amphids of this species never have been described properly, they are sketched in Figure 10, B. The opening is a somewhat irregular oval, perhaps derived from a former spiral furrow. From it a tube leads inward and backward, somewhat curved, widening farther back to an ampulla, inside of which a number of fine fibers (terminals) could easily be seen; they emerge apparently at the base of this ampulla from an opening and connect here with nerve fibers which go down to the nerve ring.

TYLENCHUS INTERMEDIUS

A single specimen of *Tylenchus intermedius* De Man was observed, a female without an egg. It was of very small size, not reaching even the minimal length given by Micoletzky (0.560 mm.). This form is of special interest because of its morphological resemblance to *T. dipsaci*. Its measurements were as follows:

$$\frac{1.6 \quad ? \quad 19 \quad ? \quad 79 \quad 91}{1.6 \quad ? \quad 2.1 \quad \quad 1.9 \quad 1.6} \text{ 0.51 mm.}$$

APHELENCHUS TENUICAUDATUS

Measurements of *Aphelenchus tenuicaudatus* De Man (fig. 10, F) were as follows:

		N.R.	End of gland				
Female 1:	4.4	14	15	22	3370	87	0.58 mm.
	2.5	3.2	3.4	?	3.2	2	
Female 2:	3.6	14	14	35	3470	90	0.62 mm.
	2.1	2.7	2.8	?	2.7	1.8	

This species was described by De Man (7) in 1895 from *Calanthes vestita* Wall. and *C. veitchii* (the latter said to be a hybrid of *C. vestita* and *C. rosea*) grown in the nursery of Veitch at Chelsea, London, England. De Man gives as measurements for the male 0.8 mm., for the female 0.95 mm., with $a = 35$ to 36, β in the male 8.5 to 9, in the female 9 to 9.5, γ in the male 11 to 15, in the female 7¾ to 8¾, rarely 10. The specimens were therefore of considerably smaller size than those studied by the Dutch scientist; furthermore, the writer has not seen a male and concludes from this that males must be less numerous than females.

Morphologically, this species is well characterized by its head end with the well-marked six lips; by its long spear, destitute of terminal bulbs (fig. 10, F); by the large medial bulb of the esophagus, which has glandular tissue anteriorly and posteriorly to a remarkable extent; by its extraordinarily long salivary gland, dorsad from the intestine; and by its pointed and elongated tail in both female and male.

The fine annulation of the cuticle has been mentioned by De Man, also the elongated tail, ending in a hairlike portion.

The lip region is well set off; papillae and amphids could not be seen in a side view.

The spear measures 22 to 26 microns and is needle shaped, without bulbs, and without the usual division into an anterior conical and a posterior cylindrical part; it is not even set off from the esophageal tube, but in the living specimens the point of junction between spear and tube can easily be detected, because the spear is stiff, whereas the esophageal tube bends. In the fixed specimens the spear is sometimes very difficult to see, but its inner end can always be detected by the ends of the protruding muscles; one or two fine indications of guiding rings were seen in the wall surrounding the spear. The esophagus, back to the oval bulb, is very slender; the bulb measures 11 to 12 by 20 to 24 microns; it is therefore about twice as long as wide. Nearly its whole anterior half consists of a granular, somewhat glandular substance, penetrated only by the esophageal tube; then follows a muscular section with the central valves, which are rather short and somewhat curved; then follows again a granular terminal portion, through which the esophageal tube connects with the intestine. Apparently connecting with the dorsal side of this granular end portion is the outlet of an extremely long single salivary gland, which extends 142 microns back to the dorsal side of the intestine. In some specimens the outlet of this gland could be seen penetrating the granular end portion of the bulb and emptying into the bulb cavity at the posterior end of the valves. This, however, was rather indistinct. The nerve ring is sometimes located close behind this bulb, in other cases only a short distance caudad.

The anterior end of the intestine is thin walled and transparent, but shows already a remarkably wide cavity, exhibiting therefore a still further advanced transformation of this formerly esophageal part into true intestinal tissue. The walls of the intestine are rather thin, and the cells contain a number of fine globules. The rectum and anal opening in the fixed material are rather obscure, but are readily seen in living material. The excretory pore in the living specimen is mostly in front of the esophageal bulb; in fixed material, however, ventrad from it.

The thin, slender ovaries are always to the right side of the intestine, and the eggs are rather small and apparently deposited in an unsegmented stage. Numerous sperms were seen in a receptacle at the end of the oviduct.

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THE OCCURRENCE OF ACETALDEHYDE IN BARTLETT PEARS AND ITS RELATION TO PEAR SCALD AND BREAKDOWN[1]

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INTRODUCTION

The opinion has long been held that functional or physiological disorders of fruits held in storage have their origin in the accumulation of certain toxic metabolites. Several organic substances which may possibly be instrumental in bringing about conditions commonly known as physiological diseases have been identified as products of metabolism. Brooks, Cooley, and Fisher (*1*),[2] who investigated apple scald, found evidence which suggested that the disease is due to volatile or gaseous substances, other than carbon dioxide, which are produced in the metabolism of the fruit and which can be carried away by air currents or taken up by various absorbents. They were able to produce effects resembling scald by exposing apples to the vapors of various esters (*2*) and demonstrated that the disease can be controlled by the use of oil-impregnated paper wrappers which are capable of absorbing the odorous emanations from the fruit (*3*). Power and Chesnut (*7*) found that the odorous constituents of the apple are the amyl esters of formic, acetic, and caproic acids, with a small quantity of the caprylic ester and a considerable proportion of acetaldehyde, and demonstrated that acetaldehyde is a product of the metabolism of the fruit, suggesting that it might be one of the factors involved in the production of apple scald.

Overholser, Winkler, and Jacob (*6*), studying the internal browning of Yellow Newtown apples, found that the disease is greatly reduced by ventilating the fruit and, during the early part of the storage season, is likewise reduced by wrapping the apples in oiled paper. They concluded that the disease is due to the accumulation of essential oils or similar deleterious substances which are produced by the apples in storage and that the internal browning of Yellow Newtown apples and apple scald are quite closely related with respect to cause.

Thomas (*9*) studied the formation of ethyl alcohol and acetaldehyde in apples and found that acetaldehyde was produced in large quantities in certain mixtures of carbon dioxide and oxygen and that high concentrations of this substance were often accompanied by browning of the cells.

The fact that acetaldehyde may occur normally and in relatively large quantities in apple tissues (*7*, *9*) suggests at once the possibility of this substance being intimately related to certain nonparasitic diseases commonly found in stored fruits.

The investigations here reported are the result of a study made upon the occurrence and accumulation of acetaldehyde as a toxic

[1] Received for publication Sept. 30, 1927; issued January, 1928.
[2] Reference is made by number (italic) to "Literature cited," p. 992.

metabolite in pear tissue and its relation to the two physiological diseases known as pear scald and pear breakdown.

Pear scald is the name applied to a brown or black discoloration of the outer tissues of the fruit. In early stages the disease is entirely superficial, but it progresses rapidly into the flesh at warm temperatures. The taste and odor of the fruit are characteristically disagreeable and sickening and can be detected in advance of the appearance of actual discoloration.

Pear breakdown or core breakdown is characterized by a brown discoloration and softening of the core tissues. The odor and taste noted in scalded pears is also characteristic of those affected with breakdown, and the two diseases frequently are associated in the same fruit.

Both scald and breakdown have become factors of major economic importance in the handling of pears since the development of modern methods of refrigeration and of the modern commercial canning industry, which involve the handling of pears in large quantities. Scald does not occur on fruit ripened out at ordinary air temperature, but is prevalent on fruit held in cold storage for an extended period, making its most rapid development after removal of the pears to a warm temperature.

EXPERIMENTAL WORK

IDENTIFICATION OF ACETALDEHYDE IN PEARS

In order to determine whether acetaldehyde is normally produced in living pear tissue, examination was made of the emanating gases of the fruit without altering the structure of the pears. By so doing, it is reasonably certain that the materials collected were substances resulting from the vital activities of protoplasm, and acetaldehyde, if present, could not possibly be of secondary origin resulting from chemical treatments or extractive agencies.

The method used in this study is a modification of the one described by Power and Chesnut (7). Twenty Bartlett pears were placed in a large glass jar provided with a sealed covering through which two glass tubes were inserted. One of the glass tubes reached to the bottom of the jar, the other to the top level of the pears. The air, which entered the vessel at the top, was first bubbled through a flask containing an alkaline solution of potassium permanganate and then through another flask containing concentrated sulphuric acid. The air passing out of the vessel was bubbled through a flask containing a saturated solution of sodium hydrogen sulphite. Air was drawn slowly through the system by means of an aspirator. This operation was allowed to proceed continuously for six days, at the end of which time the flask containing the sodium hydrogen sulphite solution was detached and the contents were made alkaline with sodium carbonate. This solution was then transferred to a distilling flask and distilled, the condenser and receiving flasks being cooled with ice. The distillate so obtained had the characteristic odor of acetaldehyde, and when subjected to qualitative tests for acetaldehyde the results were always positive and conclusive. With Schiff's reagent a pink color was quickly produced by the addition of a few drops of the distillate. It also reduced an ammoniacal solution of silver oxide, and acetic acid was formed when the distillate was oxidized with

chromic acid. Upon the addition of a few drops of strong sulphuric acid the pink color in Schiff's reagent gradually disappeared, which indicated that formaldehyde was not present. This negative test for formaldehyde was further demonstrated by using the modification of the Rimini test described by Schryver (*8*), which failed to produce the magenta color which is obtained with very minute quantities of formaldehyde.

The experiments above described present conclusive evidence that acetaldehyde is formed in metabolic processes of pears and that it is emanated in appreciable quantities. Qualitative tests indicate that acetaldehyde is the only aldehyde produced under these conditions, a fact which is in accord with the findings of Thomas (*9*) and Power and Chesnut (*7*) with apples.

QUANTITATIVE ESTIMATION OF ACETALDEHYDE IN PEAR TISSUES

If acetaldehyde production in pears is associated in any way with physiological diseases, a study of the residual aldehyde content of the tissues would be of importance as being indicative of conditions prior to and accompanying such disturbances. To determine this fact a quantitative study was made of the acetaldehyde in pears showing varying degrees of scald and breakdown.

Material Used

In the following experiments Bartlett pears were used throughout. These were picked August 12, 1926, and were placed immediately in cold storage. This date of picking represented the latter part of the harvest season at Peshastin, Wash., where the fruit for this work was obtained.

Every effort was made to insure uniformity in the selection of material for investigation. In order to reduce to a minimum any variability which might be present in a given lot of fruit, only those pears which were borne on the same tree, harvested at the same time, and stored under the same conditions were used in this work. Samples for analysis were selected on the basis of fruits showing the same visible amounts of scald and breakdown.

In obtaining composite samples for chemical analysis, from four to six pears were used. These were each cut into eight pieces, and samples of tissue from these portions were weighed. After weighing, the tissue was cut into smaller pieces and placed in a 500 c. c. distilling flask. Care was taken to exclude carpel walls and seeds from samples, and all manipulations involving the handling of cut tissues were conducted as rapidly as possible.

Method of Analysis

The quantitative method used in these experiments for determining acetaldehyde is a volumetric measure of additive compounds formed when aldehydes combine with sodium hydrogen sulphite. The aldehyde was removed from the pear tissue by steam distillation. About 1,500 c. c. of water was placed in a large round-bottom pyrex flask, through the stopper of which passed a bent glass tube leading to the bottom of the 500 c. c. distilling flask containing the sample of pear tissue. The latter flask was connected with a splash trap and thence to a Liebig condenser, which was cooled with ice water. The delivery tube of the condenser was passed through the stopper

of a large filtering flask and dipped beneath the surface of 50 c. c. of standard sodium hydrogen sulphite solution. The outlet of this filtering flask was connected by a bent glass tube to another smaller filtering flask which also contained 50 c. c. of sodium hydrogen sulphite solution. This second flask was used only as a precautionary measure to retain any aldehyde which might escape from the first flask. Both these receiving flasks were cooled with ice. After the water began to boil vigorously the flask containing the sample was connected to the steam generator and splash trap and the distillation conducted. After 1 liter of distillate was collected, which required about 90 minutes, it was found that all significant traces of acetaldehyde had been expelled from the pear tissue. The quantity of acetaldehyde present in the pear distillate was measured by titrating the sodium hydrogen sulphite in the receiving flasks against standard iodine, adding starch paste as an indicator.

With this method it is essential that control determinations be made and that the iodine and sodium hydrogen sulphite solutions be restandardized each day because of the deterioration of these reagents.

The results of quantitative studies made upon pears for acetaldehyde content are presented in Tables 1 to 7, inclusive. The analyses shown in these tables are representative of a large number of determinations made upon fruits under the physiological conditions there described. Repeated experiments all showed the same trend, but the detailed results are omitted from this paper for the sake of brevity.

ACETALDEHYDE IN NORMAL, SCALDED, AND BROKEN-DOWN PEARS

Bartlett pears were removed from cold storage. One lot was sampled immediately and analyzed for acetaldehyde, and those remaining were held for four days in a room where a temperature of 15° to 18° C. was maintained. When these pears were removed from cold storage they were hard, green, and showed no evidence of scalding. After being held for four days at 15° to 18° the fruit began to turn yellow and showed varying degrees of scald, the amount of scald varying from 1 to 10 per cent of the surface of the fruit. Preceding and accompanying the appearance of scald the characteristic disagreeable taste and odor, described in the introduction, was noticeable.

Table 1 shows the quantity of acetaldehyde present in sample tissue taken from the above-mentioned lots, expressed as milligrams of acetaldehyde in 100 gm. of fresh tissue. Composite samples were obtained from six pears of each lot. The acetaldehyde was determined as described under "Method of analysis."

TABLE 1.—*Acetaldehyde content of normal Bartlett pears from cold storage and of similar pears four days after removal from storage*

Sample	Description of sample	Acetaldehyde in 100 gms. of fresh tissue (milligrams)
No. 1	Sound green pears from cold storage (no scald)	0.3
No. 2	Pears held for 4 days at 15° to 18° C., about 1 per cent of surface showing superficial scald.	11.9
No. 3	Pears held for 4 days at 15° to 18° C., about 10 per cent of surface showing superficial scald.	17.4

The data presented in Table 1 show a rapid increase of acetaldehyde during the four-day ripening period at 15° to 18° C., and during this time scald developed to the extent there recorded.

At the end of the four-day period all fruits in these lots showed more or less scald, so that it was impossible to know whether acetaldehyde would have increased to the same extent in fruits that remained free from scald upon removal from storage.

In order to study this point, an entire box, containing 88 pears, was removed from cold storage. Six pears were taken for immediate sampling and the rest allowed to ripen at a temperature of 15° to 18° C. for a period of seven days. At the end of this time about one-fourth of the lot remained free from scald, whereas the remaining three-fourths showed varying degrees of scald, the severity ranging from slight to complete browning. Samples for acetaldehyde determinations were selected before and after the seven-day period. Analyses of these fruits are given in Table 2.

TABLE 2.—*Acetaldehyde content of scalded Bartlett pears as compared with those free from scald*

Sample	Description of sample	Acetaldehyde in 100 gms. of fresh tissue (milligrams)
No. 1	Sound green pears from cold storage (no scald)	0.3
No. 2	Pears ripened for 7 days at 15° to 18° C., remaining free from scald	1.8
No. 3	Pears ripened for 7 days at 15° to 18° C., showing about 5 per cent of surface scalded.	10.5
No. 4	Pears ripened for 7 days at 15° to 18° C., showing about 30 per cent of surface scalded.	19.5
No. 5	Pears ripened for 7 days at 15° to 18° C., showing about 100 per cent of surface scalded.*	33.7

* These pears, in addition to complete scalding, also showed a discoloration of the tissues about the core, typical of pear breakdown already described.

The data from the foregoing tables present an interesting correlation between the accumulation of acetaldehyde and the development of pear scald. In Table 2 this relation is especially striking. Pears which remained free from scald at the end of the seven-day period showed a slight increase in acetaldehyde above that in the ones analyzed immediately after removal from cold storage. However, whenever scald appeared a very marked rise of acetaldehyde occurred, and the concentration of acetaldehyde increased with scald severity.

As is noted above, pears in sample No. 5 showed evidence of breakdown in addition to being completely scalded. In view of the fact that the tissue comprising this sample showed especially large quantities of acetaldehyde, it became important to investigate the presence of acetaldehyde in pears showing breakdown only.

Table 3 presents analyses which are typical of a large number of determinations made upon sound and broken-down pears. In these experiments pears were allowed to ripen at room temperature, about 20° to 22° C., and were sampled when breakdown appeared, usually in about five days after removal from cold storage.

TABLE 3.—*Acetaldehyde content of sound and broken-down Bartlett pear tissue*

Sample	Description of sample	Acetaldehyde in 100 gms. of fresh tissue (milligrams)
No. 1	Sound hard pears from cold storage	0.3
No. 2	Soft ripe pears free from breakdown or scald	3.1
No. 3	Pears free from scald but showing breakdown at the core	23.8

The same general results were obtained in all cases, regardless of whether composite samples from a number of fruits were analyzed or whether analysis was conducted on individual fruits.

Additional confirmation of the evidence presented in Table 3 is to be found in Table 4. In these experiments the pears were removed from cold storage and kept at room temperatures for nine days. At intervals of three days, samples were taken for analysis. Care was taken to discard any fruits showing scald.

TABLE 4.—*Acetaldehyde accumulation in Bartlett pears during a nine-day ripening period at 20° to 22° C.*

Sample	Description of sample	Acetaldehyde in 100 gm. of fresh tissue (milligrams)
No. 1	Sound hard pears from cold storage	0.3
No. 2	Sound soft ripe pears 3 days after removal from storage	3.8
No. 3	Pears held for 6 days (no scald, but slight breakdown)	14.1
No. 4	Pears held for 9 days (no scald, but badly broken down)	28.1

The results in Table 4 clearly indicate a relation between acetaldehyde content and the breakdown of pear tissues similar to that which has already been shown to exist between acetaldehyde content and pear scald. These results are in accord with the conclusions of Overholser, Winkler, and Jacob (*6*) in respect to the common origin of scald and internal browning of Yellow Newtown apples.

If the accumulation of acetaldehyde in the tissues is a causative agent in producing both scald and breakdown of pears, the difference in the two disorders would then be merely one of location in the fruit. To study this further, pears were analyzed to determine the distribution of acetaldehyde in the tissues of fruits having both scalded surfaces and broken-down core areas. Samples were taken from four different regions in the fruit, namely, (1) the scalded skin surfaces, (2) normal tissue adjacent to the skin, (3) normal tissue adjacent to the broken-down core area, and (4) the broken-down core tissue.

The scalded peelings were removed with a paring knife, weighed, and immediately distilled. No effort was made to remove the white, apparently normal, tissue which adhered to the skin. The second sample, which was comprised of normal cortex tissue, was removed at a depth of about 5 mm. around the entire pear. The third sample

also contained only the normal cortex tissue removed from an area about 5 mm. from the broken-down core tissues. In the fourth sample only browned or broken-down tissue was included, and this was carefully separated from the carpel walls before weighing. As in all previous determinations, seeds were excluded from the samples. The distribution of acetaldehyde in scalded and broken-down pears is shown in Table 5.

TABLE 5.—*Concentrations of acetaldehyde in localized areas of scalded and broken-down Bartlett pears*

Sample	Description of sample	Acetaldehyde in 100 gms. of fresh tissue (milligrams)
No. 1	Scalded parings only	18.1
No. 2	Sound white tissue of scalded and broken-down pears taken 5 mm. beneath skin	15.4
No. 3	Sound white tissue adjacent to broken-down core tissue	15.7
No. 4	Broken-down core tissue	24.5
No. 5	Parings only from normal pears	1.3
No. 6	Sound white tissue from normal pears	.2

An examination of Table 5 shows that acetaldehyde accumulation takes place at the points of injury, namely, in the skin and in tissues surrounding the core. Although the white, apparently sound tissue showed a fairly high acetaldehyde content, without visible evidence of injury, it is interesting to note that despite the possibility of loss of the volatile acetaldehyde through evaporation or exhalation, it was more concentrated in the scalded tissues of the outer surface of the fruit than in the apparently sound tissues beneath. In the broken-down tissues about the core opportunity for escape of acetaldehyde was much less, and in this region it became most concentrated.

Analyses of sound pears indicated the presence of a very small quantity of acetaldehyde, but, as shown in Table 5, it is more concentrated in the skin tissues than in the cortex.

In each of the preceding experiments acetaldehyde was found in normal as well as injured tissues. Fruits fresh from cold storage had practically the same quantities in all cases. The results shown in Tables 2, 3, and 4 demonstrate that although the pears of sample No. 2 in each of these tables did not develop either scald or breakdown at the end of 7, 5, and 3 days, respectively, there was a slight increase in acetaldehyde content. From this it might be suspected that acetaldehyde may accumulate in the sound tissues of pears that are allowed to ripen with free exposure to the open air. If so, the higher concentrations of acetaldehyde in scalded and broken-down tissues might be attributed to its accumulation in sound tissues until toxic limits are reached.

To demonstrate this possibility, a study was made to determine the change in acetaldehyde taking place in pears from the time they were removed from cold storage until they had completely broken down. For this study, pears were allowed to ripen at room temperatures, 20° to 22° C., and samples were taken for chemical analysis at intervals during this time. Fruits showing visible evidence of scald or fungous decay were carefully excluded from the samples analyzed. The results of this experiment are shown in Table 6.

TABLE 6.—*Acetaldehyde content of Bartlett pears during the period of ripening*

Sample	Description of sample	Acetaldehyde in 100 gm. of fresh tissue (milligrams)
No. 1	Sound pears from cold storage	0.3
No. 2	Pears held for 5 days at 20° to 22° C	7.7
No. 3	Pears held for 8 days at 20° to 22° C	13.3
No. 4	Pears held for 10 days at 20° to 22° C [1]	23.7
No. 5	Pears held for 25 days at 20° to 22° C [2]	10.0

[1] All fruits showed core breakdown. [2] All tissues broken down; protoplasm completely disorganized.

From the data shown in Table 6, it is evident that an increase of acetaldehyde accompanies the approach to senescence and reaches its maximum concentration when breakdown of the cells becomes apparent. After 25 days the tissues were completely broken down, and the acetaldehyde content decreased markedly. From the appearance of the fruit it was evident that the cells were dead. The pears were browned or blackened throughout, and when they were broken or cut open the juice exuded freely from the cells. The decrease of acetaldehyde shown in these tissues suggests that these dead cells are either incapable of producing acetaldehyde or unable to retain it.

The progressive accumulation of acetaldehyde in normal pears when freely exposed to the air, as shown in this experiment, is not in agreement with the findings of Thomas (*9*) with apples. He did not find any appreciable increase of acetaldehyde in apples kept in air either at 1°, 15°, or 22° C. However, in all experiments conducted by the present writers with pears, increase in acetaldehyde accompanied the ripening process. The fact that apples ripen more slowly than pears may account for this difference in results. Pears ripen very rapidly when removed from cold storage and held at warm temperatures. Under these conditions of temperature, respiration proceeds at a rapid rate, and large differences in metabolic products may be measured in a comparatively short time.

In order to study the influence of temperature on the rate of maturing and on the resulting acetaldehyde content of pears, a comparison was made of sound pears taken from cold storage from time to time throughout the investigation. The cold storage referred to is a modern commercial plant, which maintains a temperature of about 0° C., and the temperature did not fluctuate more than 2° C. during the period of the present study. This comparison is presented in Table 7.

It can be seen from Table 7 that pears held at 0° C. did not increase in acetaldehyde content from October 8 to October 30, during which time the ground color remained green. However, as they gradually matured in storage, as indicated in the change of ground color from green to yellow, the acetaldehyde content showed a gradual but significant increase. It can thus be concluded that acetaldehyde is produced in the respiratory activities of pears and accumulates in the tissues as they ripen.

TABLE 7.—*Comparison of the acetaldehyde content of Bartlett pears removed from cold storage on different dates*

[After December 30, all pears began to show traces of scald]

Date of removal from cold storage	Description of sample	Acetaldehyde in 100 gm. of fresh tissue (milligrams)
Oct. 8	Sound pears from cold storage	0.3
Oct. 11	do	.3
Oct. 12	do	.3
Oct. 13	do	.2
Oct. 30	do	.3
Nov. 8	Sound pears from cold storage (ground color changing from green to yellow).	.7
Dec. 1	Sound pears from cold storage	1.3
Dec. 13	do	2.5
Dec. 30	do	3.3

The chemical origin of acetaldehyde in living tissues has not as yet been definitely established. However, its close association with ethyl alcohol, as products of alcoholic fermentation (*4*, *5*), suggests that the occurrence of these substances in plant tissues may in part be products of intramolecular respiration. Thomas (*9*) has demonstrated the presence of a zymase system in apples, this being active even though the air surrounding the apples contains large quantities of oxygen. Acetaldehyde and ethyl alcohol are among the intermediate or end products of this type of intramolecular respiration.

If then, during the life of pears, conditions of environment are such as to stimulate the production of acetaldehyde, toxicity may result after certain limits of concentration have been reached.

The presence of ethyl alcohol in pear tissues will be discussed in a later paper.

TOXICITY OF ACETALDEHYDE TO PEAR TISSUES

The toxicity of acetaldehyde to pear tissues can be demonstrated by placing fruit in contact with aldehyde gas. When placed in desiccators, over various concentrations of acetaldehyde, the pears soon develop a brown discoloration which markedly resembles the condition known as pear scald. This browning begins superficially, but gradually penetrates into the cortex until it reaches the core. The length of time required to produce the discoloration, and also the severity of the injury, depend upon the concentration of acetaldehyde to which the pears are exposed. Dilute solutions of acetaldehyde, when injected into the core cavity, cause the tissue in that area to turn brown in color and to have the appearance of typical breakdown.

The term "browning," as used throughout this paper, has reference only to visible results of injury. Browning of the tissues is a secondary action brought about by oxidizing enzymes acting upon injured or dead cells and is produced only after injury or death of the cells has taken place. Therefore, acetaldehyde is merely the indirect cause of the browning.

SUMMARY AND CONCLUSIONS

Evidence obtained in the present investigation suggests acetaldehyde as being a possible causative agent in the production of scald and breakdown in Bartlett pears. Although many workers have attrib-

uted the cause of these disorders to toxic products of metabolism, no definite work has been reported associating either pear scald or pear breakdown with the production or accumulation of acetaldehyde within the tissues.

Analyses reported in this investigation show that acetaldehyde is always found in relatively large quantities in scalded and broken-down pears. In these experiments no pears containing more than 14.0 mgm. of acetaldehyde in 100 gm. of fresh tissue, or 0.014 per cent of acetaldehyde by weight of fresh tissue, were found which did not show either scald or breakdown or both.

A positive correlation was found to exist between the severity of scald and the concentration of acetaldehyde in the tissues. A similar correlation was also found between the severity of breakdown in the fruit and the concentration of acetaldehyde in the tissues.

By analysis of pears showing both scald and breakdown, acetaldehyde was found in greater quantities in the scalded skin tissues than in the apparently normal white tissues beneath. The highest quantity, however, was found in the broken-down core areas. This distribution suggests a localization or accumulation of acetaldehyde taking place in two distinct areas, namely, the core and the outer surfaces, and that when the concentrations of acetaldehyde reach a point of toxicity in these areas, browning of the tissues occurs.

That acetaldehyde production is due to metabolic activities and is not necessarily a post-mortem phenomenon was demonstrated by the presence of acetaldehyde in the gases exhaled by sound pears, as well as by increasing quantities found in sound tissues during the period of ripening.

The disagreeable taste and odor preceding and accompanying scald and breakdown are apparently due to acetaldehyde. In extreme cases it was possible to detect a distinct odor of acetaldehyde by simply cutting the affected fruits.

The toxicity of acetaldehyde to pear tissues can be demonstrated by exposing the fruits to acetaldehyde gas or injecting dilute solutions into the tissues. It is possible that this effect may also be brought about with chemical substances other than acetaldehyde, which are present in pear tissues. However, it is doubtful whether these substances ever reach a concentration in pears sufficient to show deleterious effects.

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WOUND PERIDERM FORMATION IN THE POTATO AS AFFECTED BY TEMPERATURE AND HUMIDITY[1]

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INTRODUCTION

Largely because of its practical significance, the formation of wound cork in the potato tuber has been the subject of numerous investigations, and at present the factors governing its development are in general well understood. The present investigation had for its primary object the study of the effect of temperature on the suberization process, since the only data available are those by Priestley and Woffenden,[3] who left cut tubers for 12 days at temperatures of 15° and 25° C. and found that the rate of formation may be doubled when the temperature rises from 15° to 25° C.

MATERIALS AND METHODS

An extensive study of the relation of suberization and wound periderm in cut tubers to infection by Fusarium storage rots[4] afforded an opportunity to obtain quantities of material for a study of the effect of temperature and humidity on the wound-healing process. From the cut surface, after various intervals and exposures, tissue blocks were cut, usually about 5 by 15 by 10 mm., with the longest dimension traversing the vascular ring and extending into both cortex and inner phloem. This depth of block is sufficient to include all wound periderm that may be formed below a smooth cut. The material was fixed in ordinary chromo acetic acid and stained with an ammoniacal gentian violet. In preparing the stain it was found that the following procedure gave satisfactory results:

> To 70 c. c. of a 1 per cent gentian-violet solution in 80 per cent alcohol add gradually 30 c. c. of aqua ammonia. Stain for several hours or leave the material in the stain over night. Rinse in water and treat for a few seconds with an 8 per cent HCl solution; transfer to water and examine.

It will be found that the suberized cells and old periderm retain the violet stain; the remaining tissue, including the newly formed periderm, becomes colorless.

SUBERIZATION OF THE CUT SURFACE

The formation of wound periderm, as shown by Appel,[5] and later substantiated by Priestley and Woffenden, is preceded by a process of suberization of the cut surface. The rapidity with which this blocking off by a suberin lamella takes place depends largely on

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[1] Received for publication Aug. 8, 1927; issued January, 1928.

[2] This paper reports work carried on while the writer was a member of the Office of Vegetable and Forage Diseases.

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[5] APPEL, O. ZUR KENNTNIS DES WUNDVERSCHLUSSES BEI DEN KARTOFFELN. Ber. Deut. Bot. Gesell. 24: 118-122, illus. 1906.

environmental conditions, especially humidity and temperature. If kept at low humidity the cut surface of the tuber will dry out and crack while the tuber itself shrinks from loss of water; at high humidity, however, suberization will proceed at a rate determined largely by the storage temperature. If the temperature is very low suberization will be greatly delayed, so that noticeable changes will not be observed in the cut surface even after a period of a week or 10 days. Ultimately, however, the surface will suberize. At high humidity and high temperature suberization becomes noticeable even after the first day. It appears first in the outermost exposed cell layer. The walls in this region, as shown by the ammoniacal gentian-violet stain, retain a faint purple color, while the adjacent tissue is hyaline. As suberization progresses the coloration becomes more intense and at the same time the deeper-lying cells begin to retain the stain. The number of affected cell layers does not exceed two or three, and none of the varieties examined show the extreme depth of suberization reported by Priestley and Woffenden for some of their material. Suberization appears to be quite uniform over the entire surface. Wherever vascular bundles are exposed in the cut, the blocking off may extend farther down than elsewhere; but this may be an illusion, since the vascular tissue has a tendency to retain the violet stain, though less distinctly than the suberized cells, if the after treatment with HCl is less prolonged.

The rate of suberization for the Irish Cobbler and Russet Rural varieties is shown in Figures 1 and 2. In the Irish Cobbler variety the beginning of suberization may be seen after the eighth day at 2½° C. Russet Rural at this time is still unsuberized. At 5° suberization in the Irish Cobbler appears after the fifth day and in Russet Rural only after the eighth. At 10° in both varieties suberization is observed after the third day, at 15° after the second day, and at temperatures between 21° and 35° after only one day. Increase in temperature from 21° to 30° appears to have no effect on the rate of suberization, but in several instances at 35° suberization was found to extend less deeply. At 10° C. and above, the different varieties appear to exhibit no noticeable differences in the rate of suberization.

Olufsen,[6] in his extensive studies on wound periderm formation in the potato, states that moderate moisture is the most essential requirement for periderm formation; an excess of it plays a hindering rôle and causes cell proliferation. At the temperatures here employed no unfavorable effect was observed at the highest humidities, which at the lower temperatures closely approached saturation.

FORMATION OF THE WOUND PERIDERM

Since suberization or an equivalent blocking-off process is prerequisite to wound periderm formation, the factors governing the rate of suberization will also affect the second process. No periderm cells were observed at a temperature lower than 7° C. within the duration of this experiment. At 7° the first periderm cells appeared in the Irish Cobbler variety after the ninth day; in the Russet Rural and other varieties examined no cork cells appeared

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even on the tenth day. At 10° the first periderm cells appeared in Irish Cobbler after the fourth day, and in the other varieties after the sixth. At 15° periderm cells were seen after the third day, and at 21° and above after the second day, regardless of the variety. Once initiated, periderm formation proceeds rapidly at the higher temperatures, so that after the third or fourth day there are usually present several rows of new cells. (Fig. 3, A.)

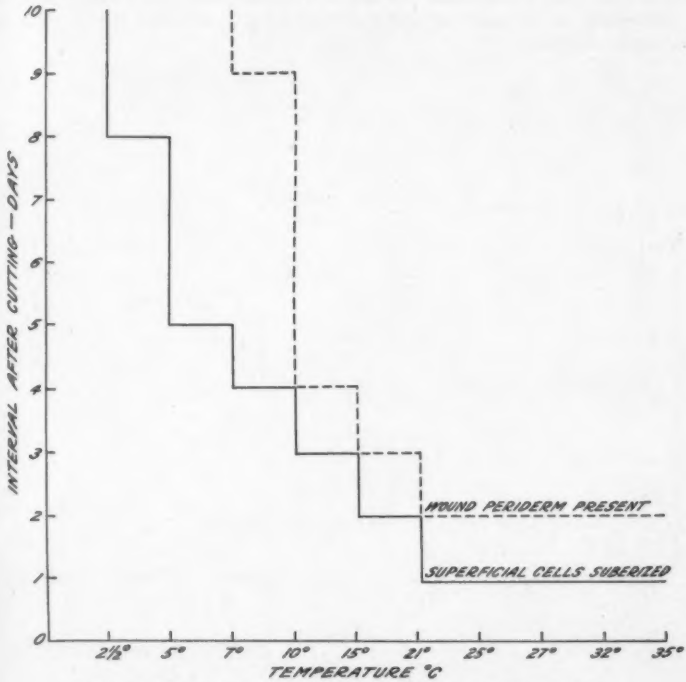

FIG. 1.—Suberization and periderm development in the Irish Cobbler variety

EFFECT OF HUMIDITY ON SUBERIZATION AND WOUND PERIDERM

Although at low temperatures variations in relative humidity produce less effect on the evaporation rate than at higher temperatures, an effect on the rate of suberization and periderm development is, nevertheless, noticeable. In tubers of the varieties Green Mountain, Spaulding Rose, and Russet Rural kept at 12° C. for six days at 64 per cent humidity, only the initial stages of suberization became evident; at 74 per cent humidity one or one and a half layers were suberized, and at 94 per cent humidity up to two layers were well suberized. Periderm formation, however, became evident only after the ninth day, and only in the third group, which had been kept at a humidity of 94 per cent.

At lower storage temperatures the effects are similar. At 6.5° C. and 70 per cent humidity, suberization was marked after a period of 53 days, but no periderm developed. At 95 per cent humidity, however, a well-developed periderm was noticeable. Different tubers often react differently. For instance, tubers of the Green Mountain variety, kept under identical conditions, showed the presence of a wound periderm in one case and its absence in another. No explanation can yet be offered for such irregularities, but their relation to infection of injuries despite apparently favorable conditions for healing is suggestive.

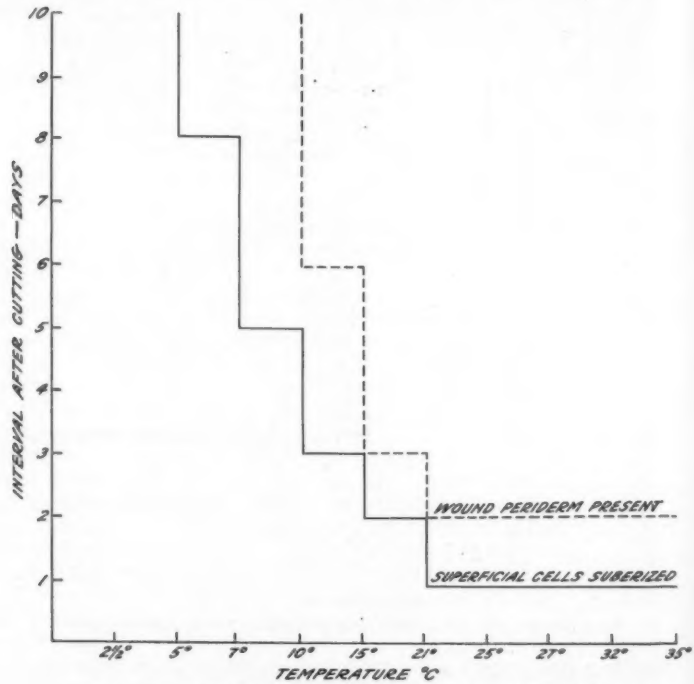

FIG. 2.—Suberization and periderm development in the Russet Rural variety

In a cross section of the tuber the topography of the periderm surface is practically flat; it may extend slightly nearer the wound surface in the vascular ring, but the difference is insignificant. The central region may sometimes show a marked contrast, exhibited in fewer periderm layers which lie farther from the surface, and this is especially true if the central medulla is very large. If the medulla is small, that is, if the phloem groups extend practically to the center of the tuber, no such regional differences will be observed. When the xylem vessels are transected, the wound periderm layer is formed around but not through them. The vessels are, however, subject to blocking by deposits of gum, which extend but a short distance back from the cut.

Concomitant with periderm formation is the disappearance of starch from the cell layers next to the cut surface. It is of interest to note, however, that the protein crystals, found abundantly in the outer cortical cells, are not used up during this process.

EFFECT OF TYPE OF INJURY ON WOUND PERIDERM

While suberization and wound cork formation in cut and stab wounds follow the method given above, tubers from which the skin

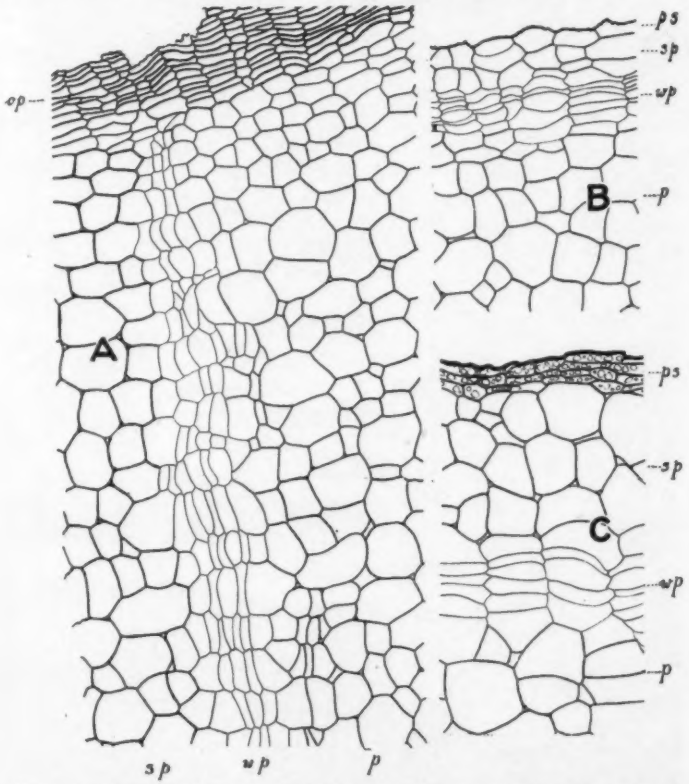

FIG. 3.—A, Wound cork formation in the Early Rose variety: *op*, old periderm; *sp*, suberized parenchyma at surface of cut; *wp*, wound cork; *p*, storage parenchyma. × 85. B, Wound cork formation in the Early Rose variety; tuber was injured by peeling off skin; peel removed smoothly: *ps*, peeled surface; *sp*, suberized parenchyma; *wp*, wound periderm; *p*, storage parenchyma. × 85. C, Same as B, except that in peeling the surface was much abraded. Note that wound periderm is formed farther away from injured surface. × 85

is peeled or which are injured by contusion show a different behavior. When young tubers are injured by blows, the young periderm cells are commonly crushed, while the older periderm shows little effect. (Fig. 4, C.) Typically there is an irregular development of periderm below the margin of the injury or wherever there is an actual break in the original periderm, but there is only slight or no development of periderm beneath the tissues which are merely crushed. (Fig. 4,

A.) It may happen that the wound periderm is formed in several tiers with crushed necrotic parenchyma cells intervening.

Tubers injured by peeling off the skin respond readily with the development of an extensive periderm. When the peel is removed

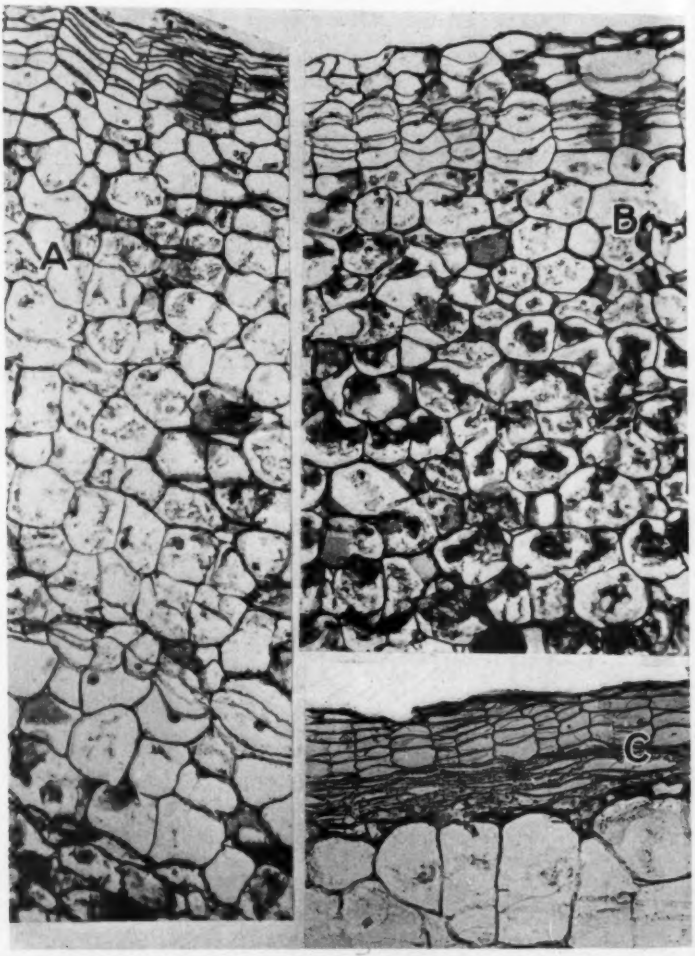

FIG. 4.—A, Deep-seated wound periderm formation in tuber injured by blows. × 85. B, Superficial wound cork formation in tuber injured by peeling off the skin smoothly. × 85. C, Young tuber injured by blows; the young periderm cells are crushed, whereas the old periderm cells are not harmed. × 85

smoothly, the new periderm cells develop within one cell layer of the surface (fig. 3, B, and fig. 4, B), whereas if the surface is much abraded, a deeper-seated periderm results (fig. 3, C).

SOME RELATIONS OF THE PHOSPHOLIPINS IN SEEDS TO OTHER CONSTITUENTS[1]

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INTRODUCTION

Since phosphorus containing lipoid substances is so universally present in plant tissues, and is so commonly found in those parts of plants in which the vital phenomena are most pronounced, it is evident that it plays an important rôle in the life processes of those tissues. Many theories have been put forward to explain its function. Overton (*22*),[3] for example, in 1901, presented the theory that each living cell is surrounded by a semipermeable membrane consisting of lipoid material, which regulates the passing into and out of the cell of substances necessary for metabolism and growth. However, present knowledge of colloidal substances indicates that Overton's lipoid membrane is not essential to a proper explanation of the migration into and out of the cell protoplasm of nutritious substances.

Other investigators have cited experimental results which appear to indicate that these lipoid substances play a very important rôle in the process of fat metabolism. Bang (*5*) has pointed out that the lipoids are probably the most labile of all the constituents which compose the colloidal system known as plant protoplasm, and, by virtue of this property, play an exceedingly important part in the metabolic processes. The same labile character which apparently makes them so important in the chemical changes in the plant tissue makes them equally unstable compounds with which to work. For this reason pure preparations for investigational purposes are very difficult to obtain, and as a result there is as yet no definite and certain knowledge of what their functions are.

REVIEW OF LITERATURE

The presence of phospholipins in plants was probably first observed by Knop (*18*), who isolated from certain plant tissue fatty extracts which contained phosphorus. This observation was confirmed the following year by Töpler (*24*). A few years later Hoppe-Seyler (*15*) isolated from peas a fatty substance very similar in nature to egg lecithin. Heckel and Schlagdenhauffen (*14*) showed that the phospholipins were of general occurrence in plants. The first definite proof of the similarity of these substances to lecithin was given by Jacobson (*17*), who showed that one of the decomposition products of these phospholipins was choline. Shortly afterwards Schulze and

[1] Received for publication Aug. 24, 1927; issued January, 1928. Published with the permission of the Director of the Oklahoma Agricultural Experiment Station.

[2] The writer wishes to thank Dr. C. T. Dowell, Prof. H. F. Murphy, Dr. V. G. Heller, and Prof. W. D. Gallup for their cooperation in the course of these investigations.

[3] Reference is made by number (italic) to "Literature cited," p. 1017.

Likiernik (*27*) obtained from seeds lecithinlike substances, which, when hydrolyzed, gave fatty acids, glycerophosphoric acid, and choline, and for a time it was believed that the only phospholipin present in plants was lecithin. But further investigation soon revealed the presence of phospholipins other than lecithin.

The investigations along this line have consisted essentially in the extraction of the fatty substances from the plant tissues with various solvents and estimating the phosphorus in the fatty extract. The amount of lecithin present was calculated from the phosphorus thus obtained. Töpler (*24*) extracted several varieties of seeds with ether and estimated the percentage of lecithin by this method. A few years later Beyer (*7*) and Jacobson (*17*) demonstrated that ether was unsuitable for the quantitative extraction of these substances. Schulze and Steiger (*28*) showed that more complete extraction could be obtained by using both ether and alcohol in the extraction process. A number of quantitative estimations were carried out, using the ether-alcohol extraction method, by Schulze and Steiger (*28*), Bittó (*9*), Stutzer (*30*), and others (*31*). The accuracy of such a method would necessitate the complete extraction of the phospholipins, and the extraction of no other phosphorus-containing substance. Bittó (*8*) criticized Schulze and Steiger's ether-alcohol extraction on the basis of incompleteness. Schulze (*26*) maintained that Bittó's criticism had but little weight, since materials which had been extracted by this method retained only traces of lipoid phosphorus. Later (*25*) he stated that it was not certain that cold alcohol would completely extract the phospholipins from plant material, but to guard against decomposition he recommended the maintenance of the extraction temperature under 60°. From time to time other investigators have adopted slight modifications of the above method. Results obtained by these methods give but a general idea of the phospholipin content of the substances examined, and are not to be regarded as absolute.

While no perfectly reliable method for the estimation of the plant phospholipins has as yet been evolved, results obtained by different investigators indicate that the leguminous seeds contain considerably more of these substances than do the cereal grains.

A number of attempts have been made to establish a relationship between the amounts of phospholipins in seeds and some of the other constituents. Schulze and Likiernik (*27*) and Schulze, Steiger, and Maxwell (*29*) found that the phospholipins varied directly with the protein content. This observation was later confirmed by other investigators. Parrozzani (*23*) made observations which led him to believe that the ratio is more definite with the amide nitrogen than with the total protein.

Thus it is apparent that the available information concerning the plant phospholipins is very meager, especially that which relates to function in the life processes of the plant.

The investigations herein reported were made for the purpose of (1) ascertaining the relative amounts of phospholipins in various seeds and determining the relation of the phospholipins to the ash, protein, fat, and total phosphorus present in the seeds; (2) to ascertain the relation of the phospholipin content to the ash, fat, protein, and phosphorus content at different stages in the development of the seeds; and (3) to ascertain the effect of germination on the com-

position of the seeds. The investigations were applied to the seeds of the grain sorghums in particular, since this group offers a closely related series when considered from the botanical standpoint, yet one whose members differ in color, size, chemical composition, and nutritive properties.

EXPERIMENTAL METHODS

For the first group of experiments 60 samples of seeds, representing different varieties of corn, beans, peas, wheat, grain sorghums, and others were secured from the agronomy department. The only data available concerning these seeds were the name of the variety and the year in which it was grown. The seeds when obtained were clean, dry, and in a good state of preservation, being free from molds, weevil, dirt, and other contaminations. From 150 to 200 gm. portions of each variety were ground in a small hand mill, until the total material passed through a 60-mesh sieve. After grinding, the samples were exposed to the atmospheric conditions of the laboratory for a time, mixed thoroughly, and preserved in glass containers. Moisture determinations were made upon each sample previous to the analysis. This determination was made by drying a 2 gm. sample to constant weight in an electric oven at 100° to 102° C.

The ash determination was made by incinerating a 2 gm. sample to dull red heat in an electric muffle. Nitrogen was determined by the Gunning modification of the Kjeldahl method. Protein was calculated by multiplying the nitrogen thus obtained by the conventional factor 6.25. The use of this factor induces a source of error, as it has been found that it varies with the different seeds. But since the factors for all the seeds involved have not been definitely established, and since most of the available data concerning the protein of these seeds have been obtained by this factor, it was used in this investigation. The fat, more properly termed "ether extract," was determined by extracting a 2 gm. sample of the dry material with anhydrous ether in a continuous extractor for 16 hours and noting the loss in weight of the sample.

The total phosphorus was estimated according to the method of the Association of Official Agricultural Chemists for the estimation of phosphorus in plant constituents (*4*). The results are expressed as phosphorus in order to permit a more direct comparison with the results obtained in the phospholipin determination.

The phospholipins were estimated by extracting the dry seeds with an ether-alcohol mixture, and estimating the phosphorus in the extracted material by the microcolorimetric method (*12*). The material was dried in an electric oven for two hours at a temperature of 100° to 102° C. before weighing out the sample.

Several modifications of the former procedure were made in accordance with the type of seeds under investigation. The amount of solvent varied from 50 to 250 cubic milliliters per gram of material, depending upon the fat content of the particular seed. After the tightly corked extraction tubes had been shaken vigorously for 10 minutes, they were placed in a warm bath maintained at 30° C., where they remained for two hours. At the end of this time the tubes were again shaken and allowed to settle. Phosphorus was determined in aliquots of the supernatant liquid. This determination

was performed in sets of 10 each, and usually three or four standards of different concentration of phosphorus were carried along simultaneously. No comparisons were made in which the standard differed more than 30 per cent from the unknown solution. For example, when a standard was set at 20, the reading of the unknown fell between the limits of 14 and 26. The results are expressed as percentage of phosphorus in the dry materials.

For ease of study and interpretation, the results of the analyses, when possible, have been grouped according to the various types of seeds and presented in table form. All determinations were made in duplicate or triplicate, and repeats made until consistent agreements were obtained. The data presented are the average values obtained by this method.

EXPERIMENTAL DATA

RELATION OF THE PHOSPHOLIPINS TO THE ASH, PROTEIN, FAT, AND TOTAL PHOSPHORUS IN VARIOUS SEEDS

The constituents of the grain sorghum seeds determined on a dry-matter basis are shown in Table 1. In considering the data here presented, a considerable variation in the percentage of the several constituents is noted. These variations are not limited to the different varieties of seeds, but occur in different samples of the same variety. Such findings indicate that the composition of a seed depends, at least to some extent, upon factors other than type and variety. It is common knowledge that both soil and climatic conditions have a definite effect upon the ash, protein, and phosphorus content of many seeds. The effect of these factors upon the fat and phospholipin in a given type of seed have not as yet been definitely established, but it stands to reason that these two components would be augmented, more or less, by the same factors. If the variations in soil composition and climatic conditions do not explain the discrepancies in the data presented, then we have no explanation to offer.

A number of investigators have determined the percentage of lipoid phosphorus in various seeds, multiplied these values by a factor, and designated the products as the percentage of lecithin in the seeds. Such a procedure must be based upon two assumptions: (1) That lecithin is the only phospholipin in such seeds, and (2) that lecithin has a constant composition. Both of these assumptions have been proved to be false, for phospholipins other than lecithin are known to exist in seeds, and purified lecithin from various seeds has been found to contain various percentages of phosphorus. This variation is readily understood when we consider the complex molecular structure of this substance and the possibility of its forming conjugated products with other substances. This discrepancy in the percentage of phosphorus may be explained in several ways. First, by any variation in the molecular weight of the fatty acids which compose the lecithin molecule. Any increase in molecular weight would result in a decrease in the percentage of phosphorus, while a decrease in molecular weight would have the opposite effect. Such variations in the fatty acids are frequently found in the more familiar glycerol esters or fats. For this reason, this type of phospholipins should not be classed as a single substance but as a class of substances having many possible members. A second explanation of this devia-

tion may be found in the fact that the phospholipins are frequently found conjugated with other substances, which results in a marked increase in molecular weight and consequently a decrease in the percentage of phosphorus. For example, a number of investigators, on finding their purified product to contain less than the theoretical percentage of phosphorus, subjected it to hydrolysis and obtained therefrom one or more molecules of carbohydrate. A third explanation lies in the fact that the complex substances such as the phospholipins are exceedingly difficult to purify and yet more difficult to preserve in a stable form after purification. Consequently the variation in the phosphorus content may be due to contamination or to products of disintegration of the originally pure substance. The contaminating substance may be phospholipin of different molecular structure than that of the lecithins, or it may be a nonphosphorus-containing substance.

TABLE 1.—*Percentage of ash, protein, fat, total phosphorus, and lipoid phosphorus in seeds of the grain sorghums, determined on a dry-matter basis, 1924 to 1926*

Seeds used	Sample No.	Year	Ash	Protein	Fat	Total phosphorus	Lipoid phosphorus
			Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Darso	1	1924	1.87	12.56	3.12	0.240	0.0310
	20	1925	2.00	11.94	3.35	.227	.0353
	66	1926	1.53	13.28	3.70	.258	.0392
	72	1926	2.12	11.88	2.76	.249	.0372
Red kafir	2	1924	1.72	12.66	3.21	.183	.0420
	18	1925	1.87	9.56	2.24	.179	.0436
	64	1926	1.55	11.72	3.24	.201	.0452
White kafir	3	1924	1.88	12.44	3.59	.214	.0330
	12	1925	1.74	12.35	2.73	.205	.0456
	73	1926	1.81	12.15	3.11	.218	.0356
White milo	4	1924	2.12	12.37	3.08	.233	.0360
	16	1925	2.22	12.06	2.39	.214	.0370
Feterita	5	1924	1.68	12.94	3.73	.288	.0260
	13	1925	1.75	12.50	2.67	.310	.0356
	67	1926	1.63	14.47	2.71	.323	.0356
Yellow milo	15	1925	2.17	10.83	2.12	.253	.0420
	71	1926	2.29	10.50	2.53	.266	.0454
	74	1926	2.14	12.56	2.90	.253	.0505
Sorgo	14	1925	1.85	11.94	2.36	.214	.0372
Reeds kafir	17	1925	1.94	13.91	2.74	.192	.0328
	70	1926	1.60	11.25	3.10	.227	.0336
Sorghum	* 19	1925	2.49	12.65	3.35	.210	.0396
Common kafir	21	1925	1.72	9.19	2.70	.201	.0356
	65	1926	1.57	12.66	3.02	.340	.0360
Hegari	68	1926	1.26	12.94	3.53	.297	.0364
Sunrise kafir	69	1926	1.64	11.75	3.37	.345	.0354

* This variety was not identified.

Thus it is apparent that any attempt to express the phosphorus-containing lipoid substances extracted from seeds or any other organic tissue as a single substance is without justification. Consequently, in this investigation the lipoid phosphorus extracted from the seeds has been estimated and this value has been designated as phospholipins. Lipoid phosphorus and phospholipins are to be considered synonymous so far as this investigation is concerned. It is true that the two terms designate entirely different substances, but in this case they bear a direct relationship to each other. And since this relationship has not been definitely established for the various phospholipins nothing can be gained by multiplying by an arbitrary factor, and a higher degree of inaccuracy results.

Considering the above facts, the values submitted are not to be considered as absolute but as indices of the relative amounts of phospholipin in these seeds.

It was thought that the phospholipin content of a given type of seed is always constant, regardless of the several factors which might affect the seeds during growth. However, this does not appear to be the case when the values obtained from the 26 samples of grain-sorghum seeds are considered.

Since the actual amounts of these substances in the seeds examined are comparatively small, the magnitude of the variations becomes apparent only when the percentage variation between the different values is considered. When this is done the percentage variation between the maximum and minimum values is found to range from 0 to about 96 per cent—a fact which is unexplainable at the present time. The phospholipin of this class of seeds does not appear to bear any definite relationship to any of the other constituents estimated.

Table 2 gives the analysis of six varieties of corn. It is to be noted that the phospholipin content of these six samples, while fairly constant, is much less than that in the grain-sorghum seeds. This fact indicates at once that the phospholipin content of seeds is at least not always proportional to the protein content. And since the fat content of the corns is higher than that of the grain sorghums, there is no apparent relationship between phospholipin and fat content, at least in this particular case. Phospholipin content appears to be independent of the color of the seeds examined.

TABLE 2.—*Percentage of ash, protein, fat, total phosphorus, and lipoid phosphorus in seeds of corn of different varieties; determinations on air-dry matter, 1925*

Variety	Sample No.	Ash	Protein	Fat	Total phosphorus	Lipoid phosphorus
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Reids Yellow Dent	22	1.53	12.18	4.63	0.214	0.0164
Silvermine	23	1.29	10.28	4.12	.205	.0142
Gehu	24	1.54	14.18	4.24	.201	.0136
St. Charles White	25	1.16	10.18	3.91	.183	.0134
Bloody Butcher	26	1.35	10.47	4.48	.196	.0145
Pop corn	48	1.60	11.50	4.75	.179	.0143

Table 3 contains the analysis of five samples of at least four varieties of wheats. The composition of this class of seeds resembles that of the grain sorghum seeds in many respects.

TABLE 3.—*Percentage of ash, protein, fat, total phosphorus, and lipoid phosphorus in seeds of wheat of different varieties; determinations on air-dry matter, 1925*

Variety	Sample No.	Ash	Protein	Fat	Total phosphorus	Lipoid phosphorus
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Kanred	38	1.93	11.94	2.26	0.314	0.0321
Harvest Queen	39	1.81	12.50	1.81	.262	.0332
Turkey Red	40	1.88	11.38	2.34	.279	.0312
Black Hull	41	2.03	14.10	2.19	.310	.0291
Wheat?	10	1.74	12.35	2.24	.279	.0272

Table 4 contains the analysis of a number of different varieties of beans. It will be readily observed that the percentage of phospholipins in this class of seeds is very much higher than in any of the seeds previously mentioned. This high phospholipin content appears to bear some relation to the high protein content of these seeds. No similarity can be observed between the phospholipin and either the ash or total phosphorus content.

TABLE 4.—*Percentage of ash, protein, fat, total phosphorus, and lipoid phosphorus in beans of different varieties, determined on a dry-matter basis, 1925*

Variety	Sample No.	Ash	Protein	Fat	Total phosphorus	Lipoid phosphorus
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Mung bean	6	4.90	26.12	11.13	0.389	0.1050
Soy?	7	4.93	35.25	18.42	.445	.1580
Soy Midwest	34	5.25	35.91	23.12	.389	.1025
Soy Manchu	35	4.80	38.03	22.66	.445	.0908
Soy Wilson	27	3.74	32.78	17.82	.332	.0944
Soy Morse	33	4.98	35.26	9.46	.419	.0780
Velvet Georgia	36	2.66	24.35	6.05	.402	.0832
Velvet Tracys	37	2.88	24.53	5.19	.389	.0834
Velvet?	8	2.52	28.10	10.22	.419	.0790
Navy bean	49	3.48	23.85	1.85	.244	.0452

The analysis of peas of five varieties is shown in Table 5. The composition of this type of seeds appears to be very constant, so far as the several constituents determined are concerned. The percentage of phospholipin present is slightly less than that found in the bean family, but is much greater than that in either grain sorghum, corn, or wheat.

TABLE 5.—*Percentage of ash, protein, fat, total phosphorus, and lipoid phosphorus in peas of different varieties, determined on a dry-matter basis, 1925*

Variety	Sample No.	Ash	Protein	Fat	Total phosphorus	Lipoid phosphorus
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Black eye	28	3.21	25.60	2.02	0.266	0.0784
Whippoorwill	29	3.59	25.81	1.56	.314	.0733
New Era	31	3.49	29.40	2.20	.279	.0784
Scotch	30	3.23	24.41	1.45	.288	.0723
Golden Vine	32	3.70	29.87	1.17	.310	.0776

Table 6 contains the percentage of the various constituents in a number of miscellaneous seeds. The point of interest in this table is the fact that cottonseed, which contains the greatest amount of phospholipin, shows less protein and less fat than the peanut seed. This again reminds us of the difficulty encountered in attempting a correlation between the various components of a given seed.

TABLE 6.—*Percentage of ash, protein, fat, total phosphorus, and lipoid phosphorus in several different kinds of seeds, determined on a dry-matter basis, 1925*

Seeds used	Sample No.	Ash	Protein	Fat	Total phosphorus	Lipoid phosphorus
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Peanut	9	2.15	33.93	48.65	0.332	0.0880
Cottonseed	11	4.78	23.50	25.20	.476	.1456
Hanna barley	42	2.68	13.12	1.80	.275	.0442
Russian Green oats	43	2.15	13.06	4.27	.266	.0388
Kanota oats	44	2.31	13.56	3.83	.258	.0348
German millet	45	4.34	10.87	3.32	.157	.0168
Timothy	46	5.34	15.60	3.63	.332	.0431
Clover	47	4.32	35.19	5.42	.271	.0506

Table 7 contains the average composition of the several types of seeds examined, arranged in the order of increasing phospholipin content. It is evident from this table that there is no relationship between phospholipin and ash content of various seeds. There appears to be a slight correlation between the phospholipin content and each protein, fat, and total phosphorus content of the various classes of seeds examined. This correlation, while more striking between the protein content than between the other constituents, does not appear to be always present. A further attempt at such a correlation should be made on a larger number of samples of different types of seeds grown upon the same type of soil and under similar climatic conditions. Such an investigation would eliminate two variable factors, which without a doubt have had a profound effect upon the results obtained in this group of experiments, namely, soil and climatic conditions.

TABLE 7.—*Average composition of the several types of seeds examined arranged in the order of increasing phospholipin content*

Seeds used	Number of samples examined	Number of varieties examined	Composition				
			Ash	Protein	Fat	Total phosphorus	Lipoid phosphorus
			Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Corn	6	6	1.42	11.47	4.34	0.196	0.0144
Millet	1	1	4.34	10.87	3.32	.157	.0168
Wheat	5	4	1.88	12.45	2.17	.289	.0306
Oats	2	2	2.23	13.31	4.05	.262	.0368
Grain sorghums	26	12	1.85	12.12	2.98	.245	.0378
Timothy	1	1	5.34	15.60	3.63	.332	.0431
Barley	1	1	2.68	13.12	1.80	.275	.0442
Clover	1	1	4.32	35.19	5.42	.271	.0506
Peas	5	5	3.44	27.02	1.68	.292	.0760
Peanut	1	1	2.15	33.93	48.65	.332	.0880
Beans	10	8	4.01	30.42	12.59	.387	.0926
Cottonseed	1	1	4.78	23.50	25.20	.476	.1456

RELATION OF THE PHOSPHOLIPINS TO THE ASH, PROTEIN, FAT, AND TOTAL PHOSPHORUS IN SEEDS OF GRAIN SORGHUMS AT DIFFERENT STAGES OF MATURITY

The investigations of Schulze and Frankfurt (*26*) led them to believe that unripe seeds contained much less phospholipins than ripe seeds. On making a study of the composition of corn and bean seeds at different stages of growth, André (*1*) found that the total nitrogen

in the dry matter increased as maturity approached, but the percentage of both ash and nitrogen decreased. André (*2*) found also that the amount of ash in lupine and haricot seeds increased with maturity, but the actual percentages decreased, while there was a definite increase in both the amount and percentage of phosphoric acid. Further investigations by André (*3*) led him to believe that the transformation of nitrogen during maturation is the reverse of that during germination. Zaleski (*34*) observed an increase in protein phosphorus with a corresponding decrease in phosphoric acid as the seeds approached maturity. The lipoid phosphorus remained practically unchanged. McClenahan (*20*) made a very complete study of the fatty substances of the black walnut at various stages of growth and the results obtained indicated a definite decrease in the phospholipin content with the approach of maturity.

From the examples cited, it is evident that there is a lack of agreement concerning the effect of the stage of maturity upon the phospholipin content of seeds. No satisfactory explanation of these discrepancies is available. One possible explanation is that the variation in the phospholipin of seeds at successive stages of growth differs with the particular seeds investigated. Analyses were therefore made to ascertain the relation of the phospholipin content of the seeds of certain members of the grain-sorghum family to the ash, fat, protein, and phosphorus content of the respective seeds at various stages of development.

Twenty-one samples, representing seven varieties of grain sorghums, were used. These samples were obtained from the agronomy department and had been grown simultaneously upon adjoining plots, on similar soils, and under similar climatic conditions. The three stages at which the seeds were gathered are designated as milk, dough, and mature. Since the head of the grain-sorghum plant does not ripen uniformly from base to apex, only such portions of each head were selected as would fall in each of these stages. Thus, samples were obtained which better represented the different stages of maturity of the different varieties than could have been procured by taking the whole heads of seeds from fewer plants. As soon as the samples were collected they were taken to the laboratory where they were freed of hulls, stems, and other adhering material. Two 100 gm. portions of each sample were weighed, placed in glass containers without tops, and dried at room temperature. When dry, the seeds were ground in a small hand mill until the total mass passed through a 60-mesh sieve, and then stored in glass containers until the desired analysis could be made.

To ascertain the moisture content of the freshly picked seeds, duplicate 10 gm. samples were placed in moisture dishes and dried to constant weight in an electric oven at 100° C. It was found that the average percentage of moisture in the fresh seeds in the milk, dough, and mature stages was 52, 40, and 29, respectively. The moisture content of each sample of the air-dry material was determined just before the analysis.

The procedure and methods of analysis were the same as those employed in the first group of experiments.

The results of the analyses have been arranged in tabular form to facilitate comparison. (See Tables 8 and 9.) The figures given

represent the average values obtained by two or more consistent determinations. Table 9 contains the same data as Table 8, except that the determinations have been made on the basis of the original material.

TABLE 8.—*Percentage of ash, protein, fat, total phosphorus, and lipoid phosphorus in sorghum seeds at different stages of maturity, determined on a dry-matter basis*

Seeds used	Sample No.	Stage	Dry matter	Ash	Protein	Fat	Total phosphorus	Lipoid phosphorus
			Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Red kafir	50	Milk	42.0	2.43	11.78	2.36	0.156	0.0261
	57	Dough	56.5	1.87	11.32	2.87	.182	.0300
	64	Mature	74.2	1.55	11.72	3.24	.200	.0452
Common kafir	51	Milk	50.0	2.11	12.07	1.94	.286	.0300
	58	Dough	66.5	1.71	12.00	2.46	.317	.0328
	65	Mature	70.5	1.57	12.65	3.02	.343	.0360
Darso	52	Milk	46.5	2.54	11.82	1.50	.129	.0271
	59	Dough	57.8	1.97	13.28	2.57	.190	.0284
	66	Mature	72.5	1.53	13.28	3.70	.255	.0392
Spur feterita	53	Milk	53.4	2.52	13.75	1.86	.238	.0198
	60	Dough	58.1	1.89	13.78	1.94	.247	.0217
	67	Mature	72.0	1.63	14.47	2.71	.323	.0356
Hegari	54	Milk	42.6	2.60	11.41	1.66	.247	.0219
	61	Dough	54.2	1.82	12.66	2.66	.249	.0261
	68	Mature	70.2	1.25	12.94	3.53	.299	.0364
Sunrise kafir	55	Milk	54.2	3.09	10.22	1.69	.251	.0256
	62	Dough	60.5	2.15	10.57	2.51	.317	.0274
	69	Mature	72.5	1.64	11.75	3.37	.347	.0354
Reeds kafir	56	Milk	48.5	2.74	10.32	1.88	.172	.0221
	63	Dough	64.2	1.88	10.44	2.96	.212	.0283
	70	Mature	68.5	1.60	10.50	3.10	.229	.0336

TABLE 9.—*Percentage of ash, protein, fat, total phosphorus, and lipoid phosphorus in sorghum seeds at different stages of maturity, determined on the fresh material*

Seeds used	Sample No.	Stage	Factor	Ash	Protein	Fat	Total phosphorus	Lipoid phosphorus
				Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Red kafir	50	Milk	0.420	1.02	4.95	0.99	0.065	0.0110
	57	Dough	.565	1.05	6.34	1.61	.012	.0168
	64	Mature	.742	1.15	8.67	2.40	.148	.0334
Common kafir	51	Milk	.510	1.06	6.04	.97	.143	.0150
	58	Dough	.665	1.13	7.92	1.62	.209	.0216
	65	Mature	.705	1.10	8.86	2.11	.240	.0253
Darso	52	Milk	.465	1.17	5.44	.69	.059	.0125
	59	Dough	.578	1.14	7.70	1.49	.110	.0147
	66	Mature	.725	1.10	9.56	2.66	.184	.0282
Spur feterita	54	Milk	.534	1.35	7.34	.99	.127	.0106
	60	Dough	.581	1.12	7.99	1.49	.143	.0126
	67	Mature	.720	1.17	10.42	1.95	.233	.0256
Hegari	54	Milk	.426	1.11	4.86	.71	.105	.0093
	61	Dough	.542	.98	6.84	1.44	.134	.0136
	68	Mature	.702	.88	9.06	2.47	.210	.0255
Sunrise kafir	55	Milk	.542	1.67	5.52	.91	.136	.0138
	62	Dough	.605	1.29	6.34	1.51	.190	.0164
	68	Mature	.725	.91	8.46	2.43	.250	.0255
Reeds kafir	56	Milk	.485	1.32	5.19	.90	.083	.0106
	63	Dough	.642	1.21	6.68	1.89	.135	.0180
	70	Mature	.685	1.09	7.82	2.11	.159	.0228

It will be observed from Table 8 that the percentage of ash in the dry matter decreased as maturity approached in all seven samples of the grain sorghum seeds examined. These results are in agreement with those obtained by André (*2*) while working with other types of seeds. Such results would naturally be expected, since the later stages of maturation of seeds is accompanied by the storing up of starches and fats and other organic and ash constituents. The per-

centage of protein (N×6.25) in the dry material shows only a very small but definite increase, indicating that the rate of synthesis of this substance follows a course almost parallel to the increase in dry matter.

There was a definite increase in the percentage of fat in the dry matter of all seven varieties as maturity approached, thus indicating a storage of these substances during the later stages of development.

While the percentage of phosphorus in the dry seeds varied considerably with the different varieties, the amount of this substance in each variety showed a definite increase as maturity approached. The fact that the percentage of ash in these seeds decreases at the same time that the phosphorus increases indicates that this substance is entering some vital combination with the organic constituents of these tissues. With a few exceptions this increase in total phosphorus appeared to run parallel with the increase in fat content.

There is a substantial increase in the percentage of lipoid phosphorus in every variety examined. While this increase is very significant, it is not sufficient to explain the total increase in phosphorus. Nor is it to be expected that it would, as Zaleski (*34*) has shown that there is an increase in protein phosphorus during the last stages of maturity. This increase in the percentage of lipoid phosphorus is slightly greater than the increase in the percentage of either fat or total phosphorus, thus indicating an exceedingly rapid synthesis of the phospholipins in the seeds during the later stages of maturity.

An examination of Table 9, which contains the percentage of these substances in the fresh seeds, shows these increases to be much more pronounced. When considering the percentage of ash in the fresh seeds, a slight decrease in five of the seven varieties is found, while the other two remained almost constant. Such findings demonstrate that these seeds acquire their maximum ash content before or by the time they reach the milk stage. This change is followed by a slight decrease in these inorganic constituents as maturity approaches.

From the same table it is noted that the percentage of protein shows a definite increase in all cases. This increase appears to run almost parallel with the increase in dry matter, thus showing that the nitrogenous materials continue to be formed or stored in these seeds until maturity is reached. The percentage of fat, of total phosphorus, and of lipoid phosphorus, each showed an even greater increase than did the protein. Such findings again would indicate a storage of these substances in the mature seeds. From the limited data obtained, no definite relationship could be established between the rates of formation or depositing of these three substances in the seeds other than the fact that all three appear to accumulate in the seeds simultaneously.

EFFECT OF GERMINATION ON ASH, PROTEIN, FAT, TOTAL PHOSPHORUS, WATER SOLUBLE PHOSPHORUS, AND PHOSPHOLIPIN CONTENT OF SEEDS OF GRAIN SOR GHUMS

The effect of germination upon the composition of various seeds has been observed by a number of investigators, but the results have not always been in agreement. In general, the amounts of phospholipins in various seeds, at different stages of germination, appears to be influenced by the condition under which germination takes place.

Schulze and Steiger (*28*) found the phospholipins to decrease when the seeds were germinated in the dark, while Maxwell (*21*) observed a definite increase in phospholipins when the seeds were germinated in the light. Similar observations were made by Zaleski (*32*), Iwanoff (*16*), Bernardini and Chiarulli (*6*), and others. But Frankfurt (*11*) obtained contradictory results when he investigated the effect of germination upon the seeds of *Helianthus annuus*, and found an increase in the phospholipins when the seeds were germinated in the dark.

Deleano (*10*) observed the fat content to remain constant during the first eight days of germination and then decrease very rapidly. Bernardini and Chiarulli (*6*) germinated the seeds of some of the cereal grains and found that both the free and the combined phospholipins increased at about the same rate until the period of chlorophyll production, after which they decreased. In the absence of light, germination was accompanied by a loss of phospholipins, chiefly of the combined form. Hart and Andrews (*13*) found germinated seeds to be rich in soluble organic phosphorus, and drew the conclusion that the organic phosphorus of each—oats, maize, and wheat—is not transformed into inorganic phosphorus during germination. Zaleski's (*33*) experiments with the seedlings of *Lupinus agustifolius* indicate that the protein-containing phosphorus and the phospholipins (chiefly lecithin) are decomposed by enzymes during germination with the production of inorganic phosphates.

Zlataroff (*35*) made an anlysis of the seeds of *Circer aritium*, after 25 days of germination and found that the protein had decreased to about one-third of its original value, but that the nuclein content remained constant. Further observations by this investigator showed that the inorganic phosphorus increased at the expense of the protein and other more soluble phosphorus-containing compounds. The phospholipins did not decrease to the same extent, but appeared to act as a reserve substance. Van Laer and Duvinage (*19*) found the total phosphorus content of barley to decrease on germination, and a definite shift of phosphorus from the husk and endosperm to the nuclei and rootlets. These authors concluded that the phosphorus change from the endosperm to the nuclei was by way of the soluble phosphates.

From the results obtained in previous investigations concerning the effect of germination upon the composition of a number of different seeds, one is led to believe that the changes in composition during germination vary with the type of seeds under investigation, and more particularly, with the conditions under which germination takes place. With these facts in mind, an investigation was started to ascertain the effect of germination upon the composition of the seeds of the grain sorghums.

The seeds of two common varieties of grain sorghum, differing in composition as widely as possible, were selected as experimental materials. These were darso and yellow milo. The experiment was so planned as to note the effect of sunlight and darkness on the germination of seeds of both varieties.

About 5 kgm. of seeds from each variety were washed free of dust, dirt, and hulls, and carefully rinsed three times with distilled water. They were then spread upon a screen and allowed to dry for two days at room temperature. At the end of that time about 2½ kgm. of the plumpest seeds were hand-picked from each variety. Twenty-

two samples of 100 gm. each from each variety were weighed to the nearest tenth of a gram, and preserved in small glass containers with screw tops. The germination was carried on in small enamel pans, about 8 inches in diameter. A perforated desiccator plate was placed in the bottom of each pan, and a piece of hardened filter paper placed upon this plate. The 100 gm. sample of seeds was spread uniformly upon the filter paper, where they were kept moist by distilled water from the bottom of the pan, by capillarity.

At the beginning of the germinating procedure, two of the 100 gm. samples from each variety were combined and reserved as the ungerminated sample. Ten of the remaining samples were put in the germinating pans, five pans being placed in a south window and five in a dark cabinet. The temperature of the room was maintained at about 32° C. by means of an electric hot plate. The period of germination varied from 2 to 10 days, the seeds being kept moist at all times by the addition of distilled water to the bottom of the pan by means of a pipette. At the end of the period of germination, the seeds were carefully raked from the filter paper into the bottom of the pan, the filter paper being pressed in order to free it of as much dissolved material as possible. The paper was then washed with a small amount of distilled water and again pressed. This process of washing the paper was repeated twice more, a small volume of water being used each time. The perforated desiccator plate was rinsed with water and removed from the pan. This precaution was taken to prevent any unnecessary loss of soluble constituents. The pan containing the seeds and the washings was maintained at a temperature of about 50° until dry. This usually required about four hours. When dry, the total contents of the pan were removed by means of a spatula and a camel's-hair brush, and placed in a glass container. This process was continued until five samples from each variety had been germinated in both light and darkness, for intervals varying from 2 to 10 days.

Since there were two cloudy days during this series of germinations, which undoubtedly would have some effect upon the experiment, and since a larger number of germinated samples would tend to decrease any error that might have been made in the germination of a particular sample, the other 10 samples from each variety were germinated in a similar manner. This time there were three and one-half cloudy days during the period of germination.

When the process of germinating the 40 samples had been completed, each of the 2 samples germinated for the same length of time and under similar conditions were combined, thus forming a total of 22 samples, including 20 germinated samples and 2 ungerminated. The seeds were then dried for four hours at 100° C. and weighed. The difference between the loss in weight of the germinated samples and the ungerminated sample of the two respective varieties, represented the loss in dry matter during the germinative process.

The samples were then ground until the total mass passed through a 60-mesh sieve, and preserved in air-tight containers until the desired analysis could be made.

The methods of analysis were the same as those employed in the earlier experiments, except the estimation of the water-soluble phosphorus, which has not previously been mentioned.

The water-soluble phosphorus determination was carried out in the following manner: Ten grams of the dry ground seeds were placed upon a small filter paper folded to fit a small funnel, and eight 25 c. c. portions of hot water were poured upon this material in succession at such a rate that each portion had passed through the filter before the succeeding portion was added. The total extract, which measured slightly less than 200 c. c., owing to the small quantity retained by the sample, was collected in 500 c. c. Erlenmeyer flasks. The flasks were immediately corked tightly and immersed in a cold-water bath to reduce the chances of hydrolysis to a minimum. When cold, 10 gm. of trichloroacetic acid was added to each sample of extract, the flasks shaken, and returned to the cold-water bath, where they remained for four hours, being shaken occasionally during this period. At the end of this time, when the proteins had been precipitated, the extracts were filtered through dry quantitative filter paper and collected in a dry 400 c. c. beaker. One hundred cubic centimeters of this filtrate was placed in a 200 c. c. porcelain evaporating dish and evaporated to dryness on a water bath. The residue in the evaporating dish was then moistened with 10 c. c. of 10 per cent solution of sodium carbonate and again evaporated to dryness. The evaporating dish was placed in an electric muffle and the contents ignited to dull redness. When the evaporating dish had cooled, the contents were dissolved in a small quantity of warm dilute nitric acid, and transferred to a 400 c. c. beaker by rinsing with distilled water. The total phosphorus content of this solution was determined by the usual gravimetric method. The results are expressed as the percentage of soluble phosphorus in the 10 gm. of sample. This process was repeated until at least two consistent values had been obtained for each sample.

The writer is aware that such a procedure is open to some criticism, but even at that, eliminating experimental errors, such a procedure should at least give comparative values for the changes during germination. This is all that can be hoped for until more is known concerning the various changes which take place during germination and more accurate means are devised to measure them.

In this investigation all determinations were made in duplicate and repeated until consistent checks were obtained.

For ease of study the results are shown in table form. (See Tables 10 and 11.)

After two days of germination the seeds appeared very much enlarged but no sprouts were visible. After four days, about 70 per cent had sprouted or showed evidence of sprouting. After six days, about 90 to 95 per cent had sprouts averaging about one-half inch in length. At this time the seeds grown in the light were larger and had a much better color than those grown in the dark. The difference in the appearance of the two groups was yet more evident at the end of the 8-day period. At the end of 10 days the sprouts from the seeds germinated in the light had reached the height of about 1½ inches and had begun to show evidence of chlorophyll formation, while the sprouts from the seeds germinated in the dark were much more irregular in height, showed no evidence of chlorophyll production, and all of the sprouts had developed a pale yellowish color.

TABLE 10.—*Percentage of ash, protein, fat, total phosphorus, water-soluble phosphorus, and lipoid phosphorus in yellow milo and darso seeds germinated in the light and in the dark, determined on a dry-matter basis*

Seeds used	Condition under which germination occurred	Sample	Days of germination	Dry matter	Ash	Protein	Fat	Total phosphorus	Water-soluble phosphorus	Lipoid phosphorus
				Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Yellow milo		A	0	93.10	1.89	11.04	3.18	0.242	0.055	0.0451
	Light	B	2	92.00	1.92	10.62	3.10	.248	.073	.0476
		D	4	90.10	1.92	9.85	2.92	.257	.081	.0572
		F	6	88.20	1.95	9.44	2.65	.254	.098	.0645
		H	8	86.40	1.96	9.03	2.44	.274	.105	.0684
		J	10	83.00	1.99	8.60	2.08	.282	.118	.0870
	Dark	C	2	89.20	1.90	10.37	2.96	.247	.071	.0471
		E	4	87.40	1.94	9.75	2.78	.251	.083	.0491
		G	6	86.50	1.96	8.97	2.57	.258	.096	.0516
		I	8	85.10	1.98	8.85	2.41	.254	.113	.0540
		K	10	84.00	1.98	8.74	2.18	.275	.126	.0624
Darso		L	0	93.50	1.42	12.69	4.05	.235	.062	.0364
	Light	R	2	92.10	1.46	12.31	3.91	.237	.069	.0384
		S	4	89.30	1.48	12.12	3.85	.240	.083	.0406
		T	6	84.00	1.54	12.10	3.71	.250	.105	.0442
		U	8	82.20	1.56	11.41	3.56	.255	.124	.0462
		V	10	81.90	1.59	11.34	3.32	.262	.138	.0503
	Dark	M	2	92.00	1.44	12.09	3.78	.242	.060	.0386
		N	4	88.10	1.48	11.16	3.58	.251	.081	.0410
		O	6	84.30	1.51	11.12	3.30	.261	.090	.0419
		P	8	82.50	1.57	11.06	3.03	.267	.119	.0428
		Q	10	82.00	1.58	11.06	3.00	.269	.134	.0442

TABLE 11.—*Percentage of ash, protein, fat, total phosphorus, water-soluble phosphorus, and lipoid phosphorus in yellow milo and darso seeds germinated in the light and in the dark, determined on the fresh material*

Seeds used	Condition under which germination occurred	Sample	Days of germination	Factor	Ash	Protein	Fat	Total phosphorus	Water-soluble phosphorus	Lipoid phosphorus
					P. ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per cent*	*Per cent*
Yellow milo		A	0	1.0000	1.89	11.04	3.18	0.242	0.055	0.0451
	Light	B	2	.9892	1.90	10.52	3.07	.245	.072	.0471
		D	4	.9677	1.86	9.44	2.86	.248	.079	.0544
		F	6	.9462	1.85	8.94	2.54	.241	.093	.0610
		H	8	.9236	1.81	8.34	2.25	.253	.097	.0632
		J	10	.8923	1.78	7.67	1.86	.252	.105	.0776
	Dark	C	2	.9569	1.82	9.92	2.83	.237	.068	.0451
		E	4	.9344	1.81	9.11	2.60	.234	.077	.0459
		G	6	.9236	1.81	8.28	2.37	.238	.089	.0477
		I	8	.9139	1.81	8.10	2.20	.241	.103	.0494
		K	10	.9032	1.79	7.89	1.97	.249	.114	.0564
Darso		L	0	1.0000	1.42	12.69	4.05	.235	.062	.0364
	Light	R	2	.9839	1.43	12.11	3.85	.238	.068	.0378
		S	4	.9518	1.41	11.54	3.69	.228	.079	.0386
		T	6	.8983	1.38	10.87	3.33	.224	.094	.0397
		U	8	.8780	1.37	10.02	3.13	.224	.110	.0406
		V	10	.8780	1.40	9.96	2.91	.230	.121	.0442
	Dark	M	2	.9840	1.42	11.90	3.72	.238	.059	.0380
		N	4	.9412	1.39	10.51	3.37	.237	.076	.0386
		O	6	.8980	1.36	9.99	2.96	.234	.080	.0385
		P	8	.8823	1.39	9.76	2.67	.236	.105	.0378
		Q	10	.8750	1.38	9.68	2.63	.235	.117	.0387

When the percentages of the various constituents in the dry material are considered, a number of changes are apparent. (Table 10.) Most conspicuous of these is the marked decrease in dry matter as the periods of germination were increased. Corresponding decreases

were observed with the two varieties of seeds under similar conditions, but the decreases were slightly less marked when germination took place in the dark. However, the rate of decrease was greater during the first few days of germination in the dark than during a similar period of germination in the light. Since precautions were taken to prevent possible losses of the soluble constituents, the loss in dry matter must be attributed to the germinative processes going on in the seeds during the germination period.

There appears to be a very slight increase in the percentage of ash in the dry matter. (Table 10.) This increase is almost proportional to the decrease in dry matter. Such a relationship is exactly what is to be expected if there had been no loss of inorganic materials by seepage.

The percentage of both the protein and the fat decreased very rapidly during the 10 days of germination. Similar observations have been made by investigators working with other seeds. There appears to be some relationship between the rate of decrease of the protein and the fat of these seeds during germination but the ratio is not definite.

The percentage of total phosphorus ran parallel with the ash, and increased, with a decrease in the percentage of dry matter. This again is to be expected as there was little chance for a loss of this substance.

The water-soluble phosphorus and the lipoid phosphorus both made an enormous increase during the 10-day period. During this time the percentages of these substances increased from approximately 20 to 130. The increase in the water-soluble phosphorus must have resulted from the splitting off of this element from some of its conjugated products, possibly the proteins. The increase in the percentage of this fraction was proportional to the increase in the percentage of lipoid phosphorus. Such findings would suggest that during germination the phosphorus in the seed passes through the water-soluble stage before forming the more vital plant tissue by combining with the lipoids.

Table 11 contains the same data as Table 10 but expressed as percentage of the original seed. These data bring out a few facts which are not so evident from Table 10. When the percentage of ash in the original material is considered, it is found to be constant throughout the several periods of germination. Slight variations are evident but these are within the limits of experimental error. This also holds true for the percentage of total phosphorus.

Experiments were conducted to ascertain the relative amounts of phospholipins in various seeds and to determine the relation of the phospholipins to the ash, protein, fat, and total phosphorus present in the seeds.

The phospholipin content of seeds was found to be as variable as many of the other constituents. The greatest variation in the amounts of these substances occurs between different types of seeds, but minor variations are apparent between different varieties of the same type or even different samples of the same variety. The phospholipin content is independent of the color and size of the seeds. In general, the highest percentages of phospholipins are found in the seeds having the highest protein and the highest fat content, but this is not always true. The total phosphorus increases with increase

of phospholipins but the relationship is not definite. Some of these irregularities could no doubt be eliminated by taking into consideration the conditions of the soil on which the seeds were grown, together with the climatic conditions during the period of growth and maturity.

The ash, protein, fat, total phosphorus, and phospholipin content of seven varieties of grain-sorghum seeds, at three different stages of growth were determined. The results obtained demonstrate very clearly a definite increase in all of the constituents, with the exception of ash, as the seeds approached maturity. The number of samples examined does not permit a definite correlation between the rate of formation of any two of these substances.

Two samples of grain sorghum seed were germinated both in light and in darkness and the percentage of several of their constituents determined at various periods of germination. It was found that the seeds lost about 10 per cent of dry-matter during 10 days of germination. This loss appeared to be slightly less when germination took place in the dark. The percentage of ash in the dry-matter increased, but the total amount of ash remained constant. The percentage and amount of both protein and fat decreased in the dry-matter in all cases. The percentage of total phosphorus paralleled that of the ash. Both the percentage and amount of water-soluble phosphorus and lipoid phosphorus increased. The increase in phospholipins, estimated as lipoid phosphorus, was almost directly proportional to the increase in water-soluble phosphorus. The lipoid phosphorus showed a greater increase when germination took place in the light. There appears to be no relationship between the increase in lipoid phosphorus and the changes in ash, protein, fat, or total phosphorus.

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RELATION OF TEMPERATURE TO RATE AND TYPE OF FERMENTATION AND TO QUALITY OF COMMERCIAL SAUERKRAUT[1]

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INTRODUCTION

The fermentation of cabbage by lactic acid-forming microorganisms is greatly influenced by several factors. Among these factors temperature is undoubtedly one of the most important. It controls the rate and kind of fermentation and thus has a marked effect on the quality of the product.

Sauerkraut packers seldom attempt to control the initial temperature of the shredded cabbage, with the result that the temperature of the different vats varies widely at different times during the packing season. During the latter part of August cabbage is often packed at 80° F. or higher, which is the temperature suggested by LeFevre (*2*)[3] for making sauerkraut. Wehmer (*6*), on the other hand, suggests that 40° is the proper temperature. This low temperature is not uncommon for cabbage shredded in November and December.

The initial temperature of the shredded cabbage largely controls the temperature of the fermentation. The temperature of the factory during fermentation has only a slight effect on the temperature of the cabbage, for the regular-size vats are large and contain 40 to 50 tons of shredded cabbage. On the other hand, it is conceivable that room temperature may have an effect on the temperature of small quantities of cabbage. Room temperature undoubtedly had an effect in the case of the top and bottom portions of the experimental vats used in this study, which contained only 8 tons of cabbage.

EXPERIMENTAL METHODS

The experiments described in this paper were arranged to study the relation of temperature to rate and type of fermentation and to the quality of sauerkraut made under factory conditions. They were conducted in a commercial sauerkraut factory near Milwaukee, Wis.

Great care was exercised to secure uniformity of material and conditions during the experiments, especially in the filling of the vats. The cabbage used was from one field, was of the same age and variety, and had been stored for about two weeks in outside ventilated piles, 90 feet long, 10 feet wide, and 6 feet high. The piles were opened along one side for the entire length, and each load

[1] Received for publication June 11, 1927; issued January, 1928. Published with the permission of the director of the Wisconsin Agricultural Experiment Station.
[2] The authors are greatly indebted to the Frank Pure Food Company, Franksville, Wis., for their generous cooperation in this work.
[3] Reference is made by number (italic) to "Literature cited," p. 1038.

removed was representative of the whole pile. The temperature of this cabbage after being cored and cut was about 40° F. It was warmed to the desired temperatures by means of steam jets placed along a chute through which the shredded cabbage fell.

Eight vats of 8-ton capacity each were filled in two series. These oval vats were 7 feet deep. The short diameter was about 9 feet and the long diameter 11 feet. Four vats were used in the first series and an equal number in the second. Their temperatures were approximately 45°, 55°, 65°, and 75° F., respectively, when filled. The second series of vats was packed four days after the first, and was as nearly as possible a duplicate of the first series. Two days were required to fill the four vats in each series.

Dividing the eight vats into two series proved another safeguard in avoiding variation in cabbage. The four vats of each series were packed simultaneously by alternating the cartloads of cut cabbage containing about 700 pounds each. This procedure made it certain that each vat contained cabbage similar to that in the others.

Three thermocouples were put into each vat, one 2 feet from the bottom and an equal distance from the side, another in the center of the vat, and another 2 feet from the top and an equal distance from the side opposite the bottom thermocouple. These thermocouples were carefully checked before being used, and readings obtained with them while in the vats were verified by means of thermometers.

Two thick-walled rubber tubes with small Büchner funnels on the ends were placed in each vat, so that a sample of juice could be drawn from the center and bottom when desired. For the first 10 days samples were drawn at 24-hour intervals, and later at more irregular intervals. From these samples the following data on bacteria, acidity, and temperature were obtained.

The number and activity of the bacteria were measured in three ways: (1) By direct counts (Breed method); (2) by determining the time of reduction of methylene blue; (3) by the acid production in the fresh cabbage juice.

Titratable acidity was measured by boiling 10 c. c. of the samples for a few seconds to expel CO_2, and then titrating with 0.1 N NaOH, phenolphthalein being used as an indicator. The hydrogen-ion concentration of each sample was determined by the colorimetric method.

Changes of temperature in different parts of the vats during fermentation were observed by means of the thermocouples.

METHODS OF ANALYSIS

CABBAGE

Representative heads were selected for chemical analysis from the inside and outside of the pile in which the cabbage was stored. These were shredded, thoroughly mixed, 20 gm. of the material were dried for 24 hours in an oven at about 110° C., and the loss in weight calculated as moisture. Total nitrogen was determined in a 10 gm. sample of cabbage by the Kjeldahl method. To determine the water-soluble constituents, 400 gm. of cabbage were thoroughly ground, filtered, and washed to a total volume of 2 liters. Nitrogen was determined in this extract by the Kjeldahl method, amino acids by

the Van Slyke (*5*) procedure, and reducing sugars by the Shaffer-Hartmann (*3*, *4*) method. Sucrose was determined by the acid-hydrolysis procedure. After the fermentation in each vat was completed, a composite sample was taken and the products of fermentation determined.

Table 1 shows the composition of cabbage from different parts of the piles used in this experiment.

SAUERKRAUT

One hundred cubic centimeters of sauerkraut juice were subjected to steam distillation after 5 c. c. of 1 N H_2SO_4 had been added. The volatile acid obtained was titrated with 0.1 N NaOH. The non-volatile acid in the residue from the steam distillation was separated and the quantity determined by the Kutscher-Steudel (*1*) method. The alcohol in 100 c. c. of sauerkraut juice was determined by neutralizing the volatile acids present and distilling off the alcohol. The latter was then oxidized with potassium dichromate to acetic acid, which was distilled off and titrated with 0.1 N NaOH. The reducing sugars present in the sauerkraut juice were determined by the Shaffer and Hartmann method.

TABLE 1.—*Composition of cabbage from different parts of piles used in experiment*

Item	Wet basis		Dry basis	
	Outside	Center	Outside	Center
	Per cent	*Per cent*	*Per cent*	*Per cent*
Moisture	92.300	90.700	0.0	0.0
Total nitrogen	.225	.242	2.93	2.60
Water-soluble nitrogen	.107	.120	1.39	1.29
Amino nitrogen	.063	.076	.82	.82
Reducing sugar	2.660	2.596	34.51	27.83
Sucrose	.717	.808	9.32	8.64

COMPOSITION OF CABBAGE

Table 1 indicates the necessity of thoroughly mixing the cabbage as was done in the eight experimental vats, in order to obtain a uniformity of raw material. The manner in which this was accomplished has been previously described. Apparently variations in composition exist between heads selected from different parts of the pile in which they were stored. The greatest difference is in the sugar content. The cabbage at the center of the pile had lost sugar through excessive respiration. These heads were much warmer than those on the outside of the pile, which indicates a more rapid rate of respiration.

CHANGES IN TEMPERATURE DURING FERMENTATION OF CABBAGE

Figure 1 shows the changes in temperature at the center of vats 1, 3, 4, and 6 at successive intervals during fermentation. Changes in the factory temperature from day to day are also shown. It is obvious that there is a rise in temperature during the first part of the

fermentation which can not be attributed to conduction from the room, since the latter is at a lower temperature than the vats. It is difficult to tell whether the heat is supplied by bacterial or plant-cell activity. During the latter period of the fermentation the temperature of the vats tends to adjust itself to room temperature. In most cases, the rise in temperature occurs when acidity is increasing most rapidly, and falls off after this rapid increase ceases. The temperature curves of Figures 2, 8, 9, and 10 illustrate this point. This rise in temperature affords some evidence that heat is supplied during the fermentation by bacterial action rather than by plant cells alone. Furthermore, the initial rise in temperature often extends over a period of eleven or twelve days. It seems very doubtful if the activity of plant cells alone could account for a rise in temperature over as long a period of time. It is interesting to note that the rise

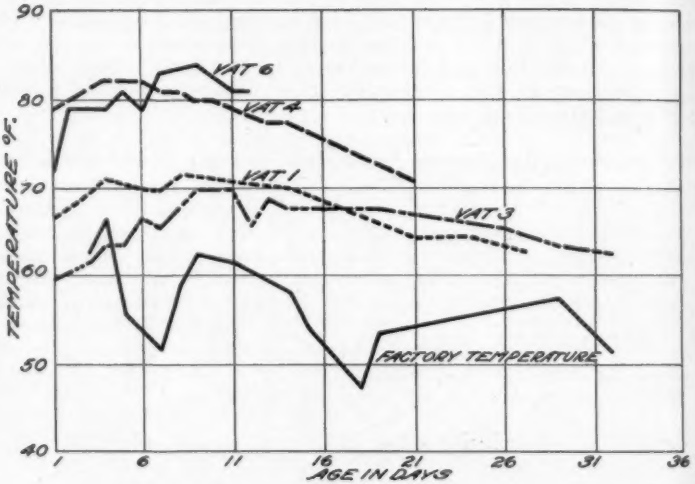

FIG. 1.—Temperature at the center of the warm vats at various times during fermentation

in temperature in all eight vats was about 10° F., and that the rate at which this rise in temperature occurred, in general, parallels the rate of fermentation (acid production).

In Table 2 are recorded the changes in temperature occurring in the three different parts of the experimental vats. It appears that the temperature at the top and bottom of each vat is more quickly affected by changes in factory temperature than that at the center. Thus the temperature at the top of vat 2, filled at 44° F., was higher than that at the center and more nearly adjusted itself to factory temperature than did the latter. In the case of the warm vat, 6, the bottom and top temperatures were the same as the center temperature at the beginning of the fermentation, but dropped off more quickly and were nearer the temperature of the factory than the latter. It should also be noted that in general the temperature at the bottom of each vat was lower than at the top. It is rather

difficult to explain this fact unless possibly it was due to convection currents carrying the heat liberated during fermentation to the surface.

TABLE 2.—*Changes in temperature of sauerkraut during fermentation*

[T = top sample; C = center sample; B = bottom sample]

Age of sauerkraut (days)	Temperature of factory (°F.)	Temperatures (° F.) in—															
		Vat 2 (initial temperature 44°)			Vat 9 (initial temperature 50°)	Vat 7 (initial temperature 52°)	Vat 5 (initial temperature 53°)			Vat 3 (initial temperature 64°)			Vat 1 (initial temperature 65°)	Vat 6 (initial temperature 74°)			Vat 4 (initial temperature 76°)
		T	C	B	C	C	T	C	B	T	C	B	C	T	C	B	C
1		41	46	44	51	50	52	55	56	61	60	66	67	77	71	75	79
2		44	45	44	51	55	55	56	58	63	61	66	68	79	79	77	80
3	63	46	44	44		56				62	62	66		79	79	79	
4	67	48	44	44	54	55	60	60	60	66	64	66	71	79	79	78	82
5	56	48	43	44		55				66	64	66		82	81		
6	54	52	45	47	55	56	62	60	60	67	67	67	70	79	79	78	82
7	52	52	45	48	55	57	63	61	59	67	66	66	70	81	83	76	81
8	59				56		63	61	61				72				81
9	63	55	47	50	57	57	62	61	60	70	70	66	72	79	84	75	80
10					57		62	61	60				71				80
11	62	56	47	50		58				67	70	65		77	81	72	
12		56	48	50		59				66		63		77	81	72	
13	60	60	50	52	58	59	63	63	61	68	69	65	71				77
14	59	60	50	52	58	60	62	64	60	67	68	63	71				77
15	55	60	51	53		60				67	68	63					
18	48	62	55	55		61				67	68	63					
19	54	60	54	53		63				66	68	62					
21					61		62	61	58				65				71
24					63		61	62	58				65				
26		61	59	55		62				63	66	60	64				
29	57	61	58	55		63				62	64	59					
32	52	60	58	55		62				61	63	58					
41	53	55	57	51	59		55	57	52								

TITRATABLE ACID AND HYDROGEN-ION CONCENTRATION

Figures 2 and 3 illustrate clearly the relationship between titratable acidity and hydrogen-ion concentration, and also the general correlation which exists between samples taken from different parts of a single vat. The changes in titratable acidity at the center and bottom of each vat are shown in Table 3. It appears from this table that the rate of acid formation is largely dependent on the temperature. It can be seen that it was necessary for fermentation to go on for 11 days in the vat whose initial temperature was 44° F. before an acidity of 0.5 per cent was reached. This period was shortened to six days in the vat whose initial temperature was 53° F., to 3 days in the 65° vat, and to 24 hours in the 76° vat. When the temperature of the shredded cabbage is between 65° and 75° the acidity increases rapidly during the first part of the fermentation, after which it increases slowly. When the temperature is below 65° the rise in acidity is more gradual and uniform throughout the entire process. These facts are clearly brought out in Figures 4 to 10, inclusive, in which changes in acidity at the center of each vat are shown. It is apparent from the data presented in Table 3 that the acidity at the bottom of each vat is about the same as at the center.

In Table 4 are recorded the results of the P_H determinations. It is apparent that these are of little value as measures of the degree of

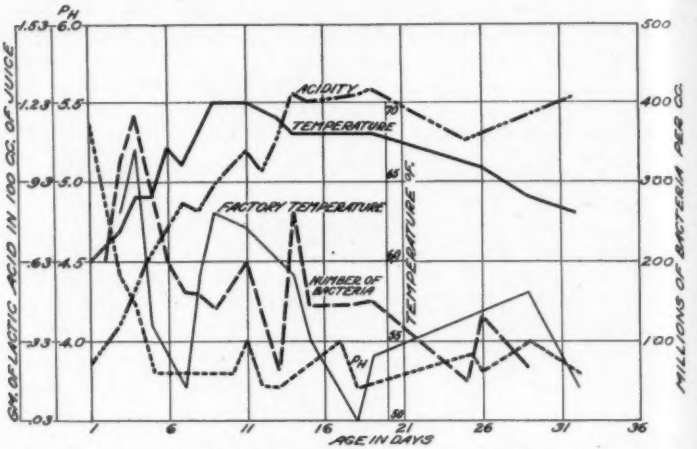

FIG. 2.—Titratable acidity, hydrogen-ion concentration, number of bacteria, and temperature at the center of vat 3 at various times during fermentation

fermentation, since in every case the P_H drops more or less rapidly, depending upon the temperature of the fermentation, to about 3.7,

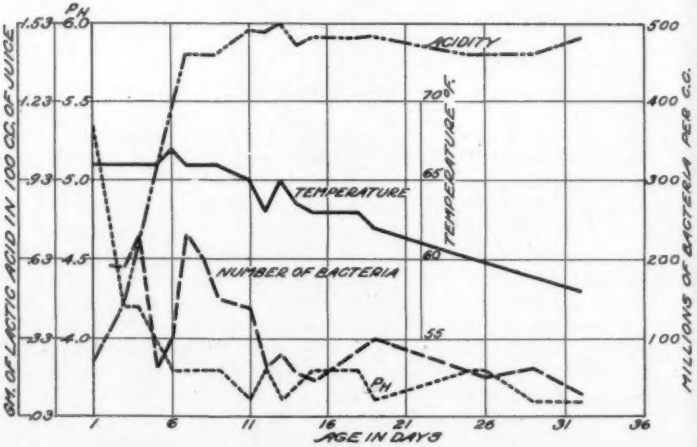

FIG. 3.—Titratable acidity, hydrogen-ion concentration, number of bacteria, and temperature at the bottom of vat 3 at various times during fermentation

and does not go any lower than this even though the titratable acidity continues to rise.

TABLE 3.—*Changes in titratable acidity of sauerkraut during fermentation*

[C = center sample; B = bottom sample]

Age of sauerkraut (days)	Percentages of lactic acid in—															
	Vat 2 (initial temperature 44° F.)		Vat 9 (initial temperature 50° F.)		Vat 7 (initial temperature 52° F.)		Vat 5 (initial temperature 53° F.)		Vat 3 (initial temperature 64° F.)		Vat 1 (initial temperature 65° F.)		Vat 6 (initial temperature 74° F.)		Vat 4 (initial temperature 76° F.)	
	C	B	C	B	C	B	C	B	C	B	C	B	C	B	C	B
1	0.17	0.14	0.09	0.09	0.15	0.14	0.09	0.16	0.18	0.23	0.37	0.19	0.38	0.52	0.59	0.64
2			.28	.23			.30	.37			.46	.26			.83	1.01
3	.15	.08	.28	.25	.39	.27	.38	.49	.40	.47	.57	.40	.87	1.06	.80	1.02
4	.18	.10	.32	.28	.61	.28	.46	.70	.54	.72	.62	.41	1.05	1.14	1.04	1.21
5	.25	.14			.64	.47			.66	.98			1.30	1.27		
6	.28	.19	.37	.46	.74	.50	.55	.82	.74	1.21	.85	.65	1.23	1.39	1.21	1.27
7	.36	.21	.42	.55	.71	.70	.53	.85	.85	1.39	.93	.70	1.24	1.53	1.31	1.32
8	.37	.26	.51	.71	.92	.74	.64	1.01	.82	1.38	1.14	.74	1.40	1.63	1.38	1.55
9	.39	.38	.58	.68	1.11	.85	.67	1.07	.92	1.39	1.16	.73	1.41	1.55	1.59	1.55
10			.67	.75			.81	1.09			1.33	.85			1.63	1.56
11	.55	.52			1.05	.95			1.05	1.51	1.35		1.36	1.63		
12	.60	.52			1.11	1.03			.97	1.50			1.15	1.64		
13	.70	.63	.84	.88	1.21	1.11	1.07	1.13	1.09	1.54	1.38	.87			1.68	1.68
14	.66	.68	.81	.85	1.09	1.13	1.13	1.13	1.26	1.45	1.53	1.04			1.72	1.57
15	.72	.64			1.06	1.21			1.23	1.48						
18	.75	.77			1.06	1.27			1.25	1.47						
19	.82	.76	1.13	1.05	1.30	1.29	1.31	1.33	1.28	1.48	1.56	.91			1.80	1.66
21			1.03	1.05			1.29	1.33			1.57	1.06			1.76	1.72
25	.91	.90	1.32	1.11	1.32	1.43	1.40	1.42	1.09	1.41						
27			1.41	1.24	1.48		1.46	1.42			1.49	1.20				
29	.97	1.06			1.31	1.46			1.19	1.42						
32	1.03	1.17			1.31	1.40			1.25	1.47						
41	1.31	1.59	1.64	1.17			1.66	1.62								

TABLE 4.—*Changes in the P_H values of sauerkraut during fermentation*

Age of sauerkraut (days)	P_H values in center of—								
	Vat 2 (initial temperature 44° F.)*		Vat 9 (initial temperature 50° F.)	Vat 7 (initial temperature 52° F.)	Vat 5 (initial temperature 53° F.)	Vat 3 (initial temperature 64° F.)	Vat 1 (initial temperature 65° F.)	Vat 6 (initial temperature 74° F.)	Vat 4 (initial temperature 76° F.)
	C	B							
1	5.8	5.8	5.9	5.8	5.9	5.4	5.7	5.4	5.5
2			5.2		5.0		4.4		4.6
3	5.9	5.9	4.6	5.8	4.3	4.4	4.0	4.2	4.0
4	5.8	5.9	4.2	5.5	4.0	4.2	3.8	4.2	3.8
5	5.4	5.6		5.2		3.8		3.8	
6	5.3	5.4	4.2	4.5	4.0	3.8	4.0	3.8	3.8
7	5.0	4.8	3.9	4.4	4.0	3.8	3.8	3.8	3.7
8	4.6	4.4	4.0	4.2	3.8	3.8	3.7	3.6	3.5
9	4.2	4.2	4.2	4.0	3.8	3.8	3.6	3.6	3.5
10			4.0		3.9	3.8	3.6		3.5
11	4.2	4.2		4.0		4.0		4.0	
12	4.0	4.0		3.7		3.7		3.8	
13	4.0	4.1	3.9	3.9	3.8	3.7	3.6		3.6
14	4.2	4.2	3.9	3.9	3.8	3.8	3.7		3.8
15	4.2	4.2		3.9		3.9			
18	4.0	4.0		3.8		4.0			
19	4.2	4.0		3.8		3.7			
25	4.0	3.8	3.6	3.8	3.6	3.9	3.6		3.5
26	4.0	3.8		3.8		3.8			
29	3.9	3.8		3.8		4.0			
32	4.0	3.6		3.7		3.8			

* For comparison the P_H value of bottom sample is given.

Additional evidence concerning this question was obtained by an analogous experiment in the laboratory. It was found that the P_H of fresh cabbage juice is 5.9. When the juice was saturated with

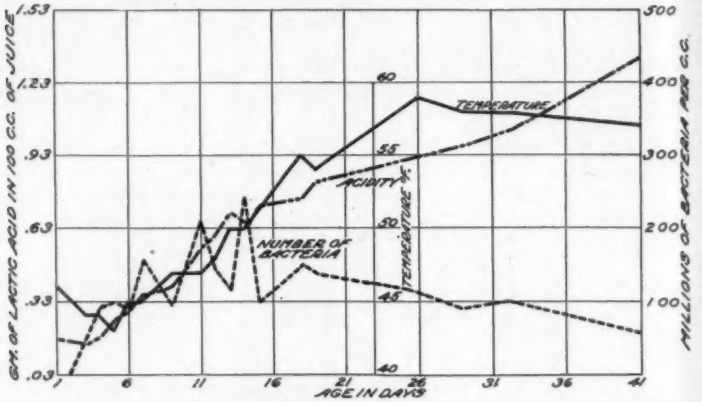

FIG. 4.—Temperature, acidity, and number of bacteria at the center of vat 2 at various times during fermentation

carbon dioxide, the P_H was lowered to 4.8. Large quantities of carbon dioxide are liberated during the first few days of fermentation of cabbage, which must almost completely saturate the juice ex-

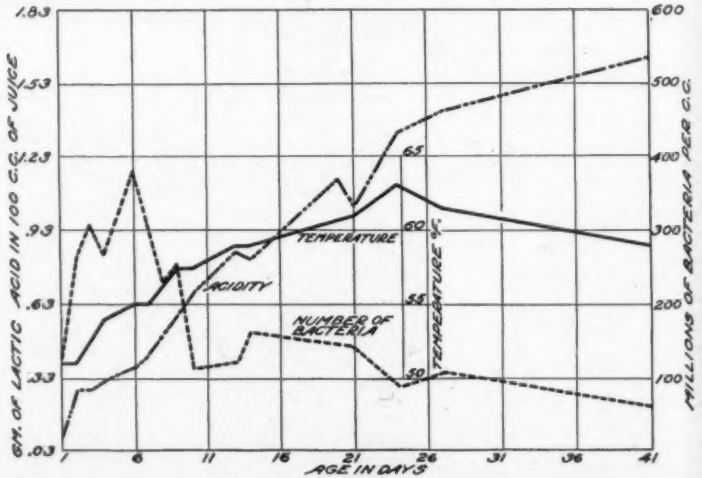

FIG. 5.—Temperature, acidity, and number of bacteria at the center of vat 9 at various times during fermentation

pressed from the latter. This would, in a large part, account for the rather sudden drop in P_H observed during the early days of fermentation before much acid had been formed.

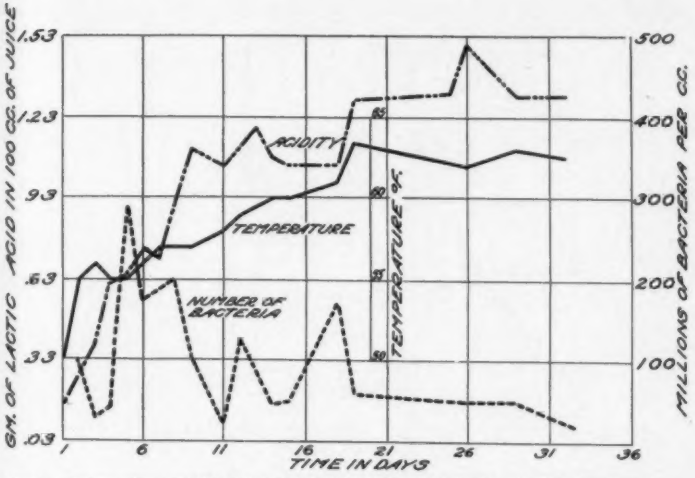

FIG. 6.—Temperature, acidity, and number of bacteria at the center of vat 7 at various times during fermentation

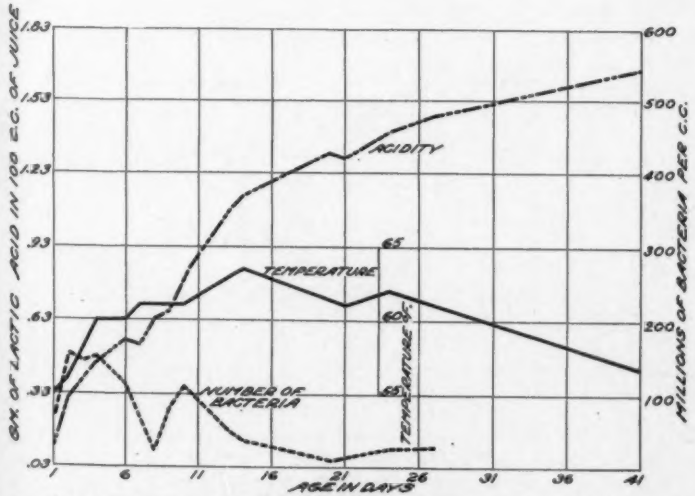

FIG. 7.—Temperature and acidity at the center of vat 5 and number of bacteria at the bottom of vat 5 at various times during fermentation

If 0.5 c. c. of 1 N. acetic acid and 1.3 c. c. of 1 N lactic acid are added to 15 c. c. of cabbage juice, a mixture is formed which has a P_H of 3.6 and contains quantities of the two acids equivalent to that

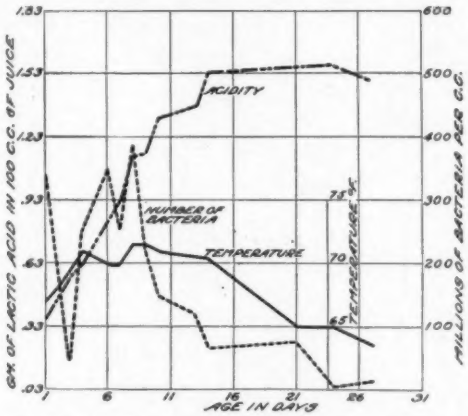

FIG. 8.—Temperature, acidity, and number of bacteria at the center of vat 1 at various times during fermentation

found in completely fermented cabbage. If the same quantities of these two acids are added to water, in which case no buffer effect is possible, the mixture will have a P_H of 2.9. These values indicate the buffering property of cabbage juice. If 0.25 c. c. of 1 N acetic

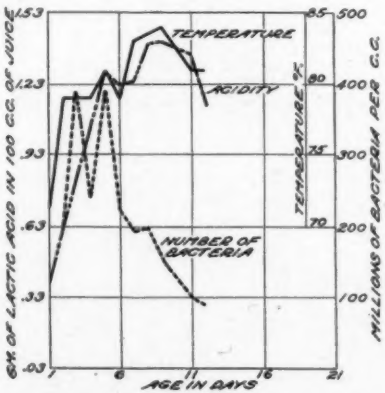

FIG. 9.—Temperature, acidity, and number of bacteria at the center of vat 6 at various times during fermentation

acid and 0.65 c. c. of 1 N lactic acid are added to 15 c. c. of cabbage juice, a mixture is formed having a P_H of 3.6 and containing quantities of the two acids equivalent to that found in half-fermented cabbage.

Since reducing the amount of acids one-half has no effect on the P_H, it is clear that P_H determinations are of but little value as a measure of changes in acidity.

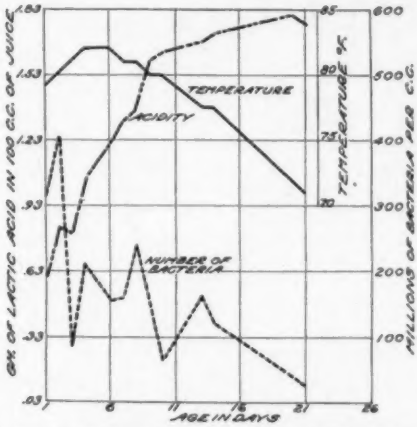

FIG. 10.—Temperature, acidity, and number of bacteria at the center of vat 4 at various times during fermentation

TABLE 5.—*Changes in number of bacteria in sauerkraut during fermentation*

[C=center sample; B=bottom sample]

Age of sauerkraut (days)	Number of bacteria (millions per c. c. of juice) in—															
	Vat 2 (initial temperature 44° F.)		Vat 9 (initial temperature 50° F.)		Vat 7 (initial temperature 52° F.)		Vat 5 (initial temperature 53° F.)		Vat 3 (initial temperature 64° F.)		Vat 1 (initial temperature 65° F.)		Vat 6 (initial temperature 74° F.)		Vat 4 (initial temperature 76° F.)	
	C	B	C	B	C	B	C	B	C	B	C	B	C	B	C	B
1			101	6			200	48			339	157			306	114
2	6	8	258	204	97	24	210	154	196	193	197	82	199	196	405	404
3	40	45	307	201	29	27	211	144	322	190	46	99	392	82	83	261
4	89	28	266	215	41	65	173	150	386	230	253	161	240	183	213	197
5	97	111			287	90				62			391	299		
6	86	51	374	207	175	115	240	113	201	105	351	122	225	190	156	218
7	158	31	303	142	189	150	190		163	235	248	21	191	188	161	301
8	123	101	229	262	205	62	73	22	159	205	389	52	200	147	240	154
9	95	81	255	203	101	80	84	76	140	152	225	39	156	82	164	124
10			112	56			61	111			152	49			61	119
11	211	94			18	79			199	137			98	14		
12	141	44			124	199			121	62			88	40		
13	117	62	121	108	88	55	62	54	64	80	121	19			162	179
14	241	61	163	139	48	78	33	37	257	57	62	42			120	31
15	97	38			49	54			143	46						
18	155	153			174	42			146	87						
20			143	143	60	32	30	12	151	109	77	22			26	14
24	116	63	88	164	50		28	26	47	60	9	63				
27			109	187			34	29	115	52	12	64				
29	91	119			50	25			63	60						
32	102	82			26	13				28						
41	55	141	63	209												

NUMBERS AND KINDS OF BACTERIA

Table 5 and Figures 2 and 4 to 10, inclusive, record the changes in the number of bacteria in each vat during the fermentation. Table 5 includes both bottom and center samples, while the curves give only the changes occurring at the center of each vat. In addition, the curves show the relation between acidity and numbers of bacteria in each vat. From the latter it appears that changes in acidity and numbers of bacteria occur more rapidly in those vats which were filled at the higher temperatures. With the exception of the 40° F. vat there are at least two high points in the bacterial curves. From this fact and also from microscopical evidence it is apparent that there is a sequence of microorganisms taking part in the fermentations. Figure 11, which represents the fermentation in a 10-gallon jar of cabbage kept at 70°, shows this sequence even

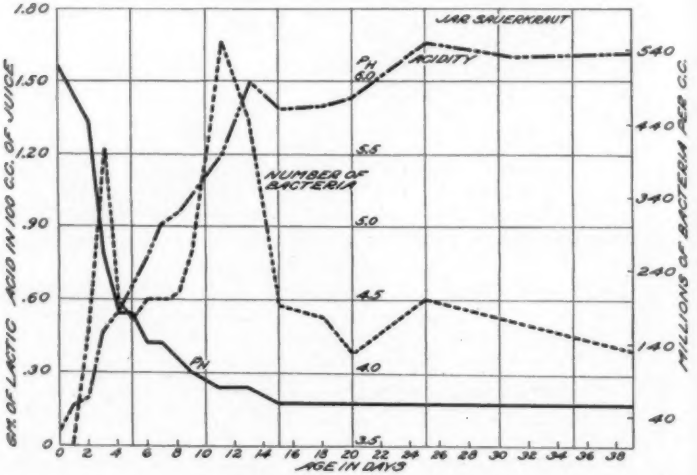

FIG. 11.—Acidity, hydrogen-ion concentration, and number of bacteria in a 10-gallon jar of fermenting cabbage

more clearly. At the first point showing a high number of bacteria, the flora consists of short stubby rods, while at the second point the flora is made up largely of long rods. There are undoubtedly additional types of organisms taking part in the fermentation which are fewer in number and less conspicuous. For example, there are the streptococci occurring during the first few days of fermentation, and the yeast, the importance of which is more or less uncertain.

It is clear that there are at least two types of bacteria taking part in the fermentation. The first is unable to withstand as high concentrations of acid as the second, and is therefore replaced by the latter.

It is worthy of note that although the number of bacteria may rise and fall throughout the fermentation, the increase in acidity is quite uniform. From this it appears that although the organisms die

their enzymes are still active, or else that the new flora which develops upon the death of the old maintains the uniform rate of acid production.

TABLE 6.—*Changes in time of reduction of methylene blue by sauerkraut during fermentation*

[C=center sample; B=bottom sample; a=not reduced in two hours]

Age of sauerkraut (days)	Time of reduction in minutes in—															
	Vat 2 (initial temperature 44° F.)		Vat 9 (initial temperature 50° F.)		Vat 7 (initial temperature 52° F.)		Vat 5 (initial temperature 53° F.)		Vat 3 (initial temperature 64° F.)		Vat 1 (initial temperature 65° F.)		Vat 6 (initial temperature 74° F.)		Vat 4 (initial temperature 76° F.)	
	C	B	C	B	C	B	C	B	C	B	C	B	C	B	C	B
1	a	a	a	a	a	a	a	15	80	80	8	9	75	75	8	8
3	a	a	40	40	20	20	40	40	5	5	25	40	5	5	40	40
4	a	a	13	13	12	12	13	95	8	8	34	120	10	10	120	75
5	40	a	----	----	40	45	----	----	25	28	----	----	35	35	----	----
6	11	11	10	10	10	9	20	a	10	10	10	a	8	8	a	20
7	----	----	12	12	----	----	27	27	----	----	27	45	----	----	45	45
8	40	40	10	10	----	----	55	a	40	40	45	a	----	----	a	a
9	22	120	15	90	120	120	a	a	120	75	50	a	120	120	a	a
10	----	----	23	65	----	----	a	a	----	----	50	a	----	----	a	a
11	20	a	----	----	a	30	----	----	10	10	----	----	a	a	----	----
12	20	45	----	----	45	20	----	----	50	27	----	----	----	45	----	----
13	7	a	25	25	a	7	105	a	a	28	a	a	----	----	a	a
14	15	a	40	a	a	50	a	45	a	a	a	a	----	----	a	a
15	15	a	----	----	a	23	----	----	a	65	----	----	----	----	----	----
18	15	a	----	----	----	a	----	----	a	45	----	----	----	----	----	----
19	40	a	----	----	----	8	----	----	a	45	----	----	----	----	----	----
20	----	----	a	25	----	----	a	40	----	----	a	a	----	----	a	a
21	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----
24	----	----	45	30	----	----	a	45	----	----	75	75	----	----	----	----
25	40	a	----	----	a	40	----	----	a	25	----	----	----	----	----	----
26	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----
27	----	----	105	105	----	----	a	135	----	----	a	135	----	----	----	----
29	75	75	----	----	a	120	----	----	a	45	----	----	----	----	----	----
32	----	165	----	----	a	105	----	----	a	150	----	----	----	----	----	----
34	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----
39	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----
41	120	120	15	40	----	----	120	a	----	----	----	----	----	----	----	----

In Table 6 are recorded the changes in time of reduction of methylene blue of bottom and center samples taken at successive intervals during the fermentations. Figures 12 and 13 show the correlation which exists between the time of reduction of methylene blue and the total number of bacteria in the center samples of two vats. A cold and a warm vat are selected as examples of this relationship, and it is apparent in both cases that when the number of bacteria is high the time of reduction of the dye is short. This inverse relationship seems to indicate that the bacteria shown in microscopic preparations are practically all living organisms.

TABLE 7.—*Changes in acid-forming ability of organisms in sauerkraut during fermentation expressed in percentage of lactic acid*

[C=center sample; B=bottom sample]

Age of sauerkraut (days)	Percentages of lactic acid in—															
	Vat 2 (initial temperature 44° F.)		Vat 9 (initial temperature 50° F.)		Vat 7 (initial temperature 52° F.)		Vat 5 (initial temperature 53° F.)		Vat 3 (initial temperature 64° F.)		Vat 1 (initial temperature 65° F.)		Vat 6 (initial temperature 74° F.)		Vat 4 (initial temperature 76° F.)	
	C	B	C	B	C	B	C	B	C	B	C	B	C	B	C	B
1	0.33	0.35	0.33	0.49	0.30	0.19	0.32	0.38	0.30	0.32	0.04	0.04	0.30	0.28	0.35	0.10
2	.37	.36	.47	.48	.30	.37	.62	.50	.40	.41	.22	.05	.42	.46	.42	.49
3	.38	.33	.44	.44	.18	.07	.47	.39	.29	.43	.05	.02	.24	.42	.44	.35
4	.18	.13	.05	.07	.17	.07	.06	.06	.21	.28	.03	.16	.24	.19	.13	.28
5	.46	.34	.....	.....	.07	.03	.....	.....	.33	.45	.....	.....	.35	.05	.....	.....
6	.45	.36	.05	.05	.11	.04	.....	.....	.35	.47	.....	.....	.43	.28	.09	.05
7	.38	.38	.06	.05	.05	.04	.02	.03	.36	.43	.06	.13	.35	.44	.10	.16
8	.01	.13	.19	.00	.23	.14	.06	.08	.12	.17	.02	.02	.14	.15	.12	.00
9	.05	.00	.23	.30	.17	.09	.00	.00	.30	.05	.00	.00	.27	.14	.24	.00
10	.....	.....	.34	.36	.....	.....	.29	.23	.....	.....	.09	.05	.....	.....	.36	.33
11	.05	.....	.....	.....	.14	.11	.....	.....	.02	.03	.11	.22	.13	.38	.....	.....
12	.16	.23	.....	.....	.02	.04	.....	.....	.23	.24	.....	.....	.....	.....	.....	.....
13	.34	.39	.....	.....	.33	.23	.29	.29	.20	.33	.....	.....	.....	.....	.....	.....
14	.....	.....	.....	.....	.42	.35	.....	.....	.37	.36	.....	.....	.....	.....	.....	.....

ACID PRODUCTION

In Table 7 are recorded the data for all of the experimental vats, from which samples were taken from day to day and added to fresh cabbage juice. The acid formed in this juice was titrated at the end of a week. When typical examples of these data are plotted, as shown in Figures 14 and 15, it is apparent that there are two points at which the ability of the samples to produce acid is at its highest. These points correspond closely with the high points in the curves showing the number of bacteria.

TABLE 8.—*Composition and grading of sauerkraut from experimental vats (wet basis)*

Age, composition, and quality of sauerkraut	Vat 2 (initial temperature 44° F.)	Vat 9 (initial temperature 50° F.)	Vat 7 (initial temperature 52° F.)	Vat 5 (initial temperature 53° F.)	Vat 3 (initial temperature 64° F.)	Vat 1 (initial temperature 65° F.)	Vat 6 (initial temperature 74° F.)	Vat 4 (initial temperature 76° F.)
Age in days	47	42	39	41	32	28	12	22
Moisture (per cent)	90.7	90.9	90.9	91	90.7	90.5	90.5	91.4
Acetic acid (per cent)	.337	.367	.378	.355	.346	.322	.266	.295
Lactic acid (per cent)	1.242	1.553	1.335	1.367	1.218	1.311	1.062	1.506
Titratable acid (per cent)	1.377	1.580	1.494	1.660	1.575	1.421	1.410	1.784
Ethyl alcohol (per cent)	.511	.528	.742	.487	.411	.320	.208	.598
Reducing sugar (per cent)	.08	.56	.20	.53	.44	1.05	.76	.35
Quality	Very good.	Excellent.	Very good.	Excellent.	Poor.	Poor.	Fair.	Fair.

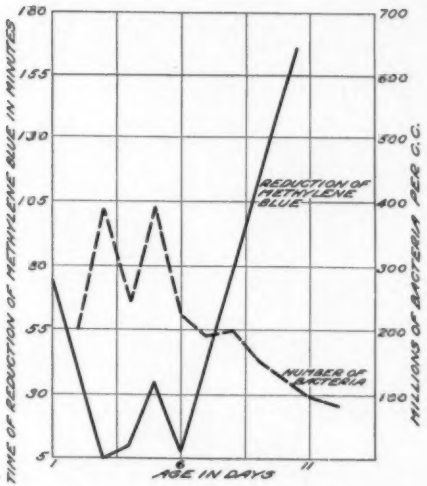

FIG. 12.—Time of reduction of methylene blue and number of bacteria at the center of vat 6 at various times during fermentation

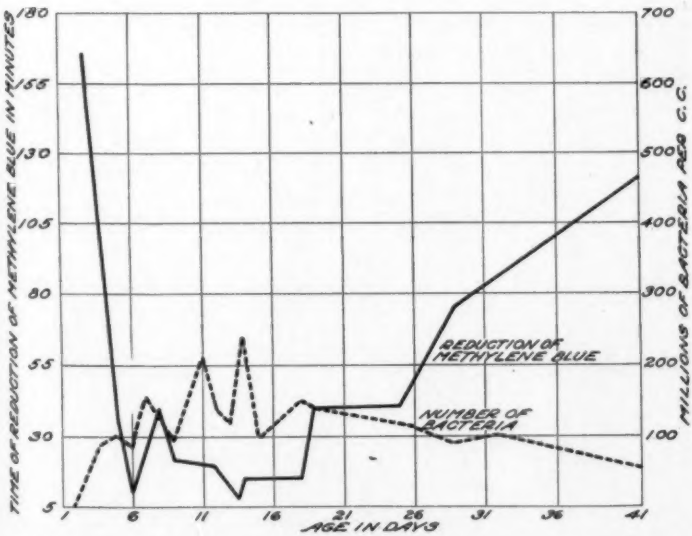

FIG. 13.—Time of reduction of methylene blue and number of bacteria at the center of vat 2 at various times during fermentation

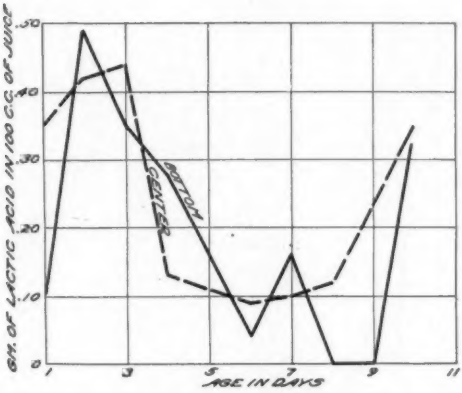

FIG. 14.—Acid-producing ability of samples obtained from the center and bottom of vat 4 at various times during fermentation

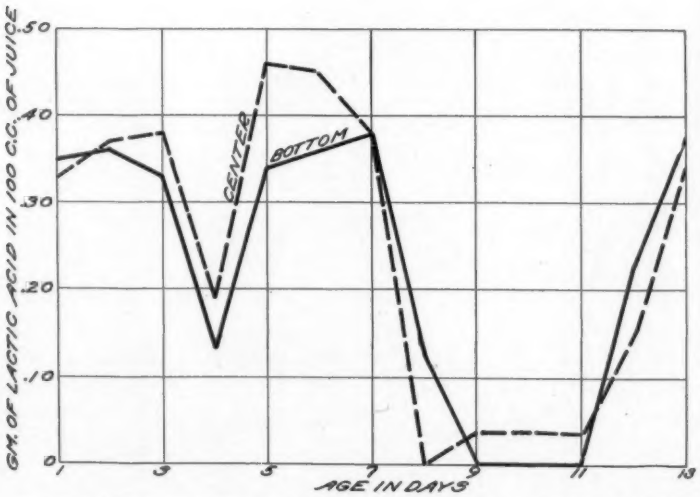

FIG. 15.—Acid-producing ability of samples obtained from the center and bottom of vat 2 at various times during fermentation

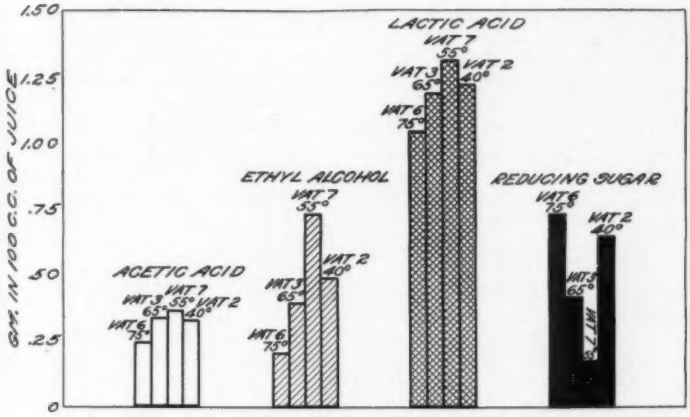

FIG. 16.—Principal fermentation products in sauerkraut obtained from vats 6, 3, 7, and 2

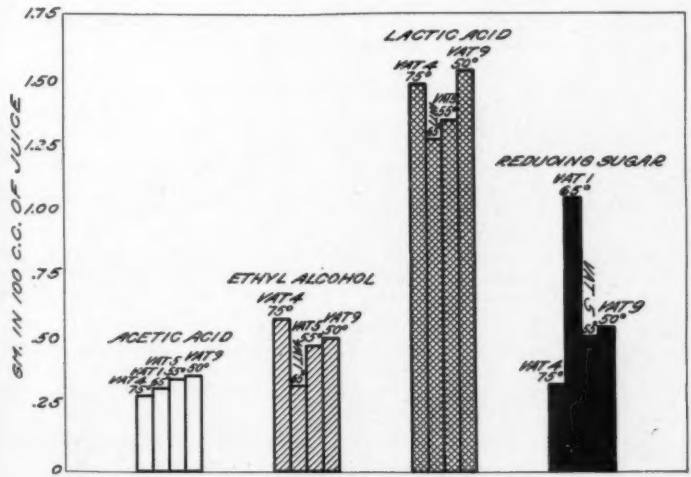

FIG. 17.—Principal fermentation products in sauerkraut obtained from vats 4, 1, 5, and 9

COMPOSITION AND QUALITY OF SAUERKRAUT

Figures 16 and 17 and Table 8 show the composition of the experimental sauerkraut in the two series of vats. The best sauerkraut was obtained from the vats at the intermediate temperatures; at higher temperatures the product was more or less pink and of poor quality. Presumably the fermentation at these temperatures went on too rapidly, affording conditions favorable for the growth of pink yeasts. It is difficult to observe any relation between composition and quality of sauerkraut from the data at hand. Undoubtedly the flavor is influenced by minute quantities of unidentified products of fermentation. As is to be expected in almost every case the amount of fermentation products formed is inversely proportional to the residual sugar.

SUMMARY AND CONCLUSIONS

Evidence is presented which indicates that heat is produced during the fermentation of cabbage.

The rate of acid production in fermenting cabbage is largely dependent upon the temperature of the fermentation. The higher the temperature of fermentation the greater the rate of acid production.

Titratable acidity is a much better measure of the degree of fermentation than are P_H determinations.

The fermentation of cabbage is carried on by a sequence of flora, consisting of at least two distinctly different types of lactic acid-producing bacteria.

The time of reduction of methylene blue is of value as an indication of the number of live bacteria in fermenting cabbage.

Sauerkraut of the best quality was produced when the maximum temperature of fermentation was about 65° F.

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STRAIN VARIATIONS AND HOST SPECIFICITY OF THE ROOT-NODULE BACTERIA OF THE PEA GROUP[1]

By G. E. Helz, I. L. Baldwin, and E. B. Fred, *University of Wisconsin*

INTRODUCTION

Since early in the study of the root-nodule bacteria, observations have been made indicating that variations may exist between strains of bacteria within a cross-inoculation group, and that such differences may be closely related to the nitrogen economy of the plant. The generally accepted opinion, however, has been that the strains of bacteria of a cross-inoculation group are practically identical as to their ability to aid the plant in the symbiotic fixation of nitrogen. Cultures distributed by public agencies and commercial concerns have been selected on the basis of the cross-inoculation group. Probably some strains are equally good on more than one species and possibly on all the members of their group, but there seem to be other strains of the nodule organism which show a specificity for a single host species, and which are of great benefit to one member of the host group, but of relatively little benefit to another member.

Even though certain strains show a preference for a single host species, it is scarcely probable that all of the strains isolated from that species would exhibit the same peculiarity. When an organism is isolated from a host plant it is usually impossible to determine its previous history, and it is possible that a strain isolated from a pea nodule may have been associated with vetch or some other host of the cross-inoculation group within a few previous generations.

REVIEW OF LITERATURE

As early as 1879, Frank (*5*)[2] recognized two types of inoculation on peas, one type forming nodules with albuminous contents, the other forming nodules containing amylodextrin. The albuminous ones contained 6.93 per cent of nitrogen in the dry matter as compared to 4.82 per cent of nitrogen in the dry matter of those containing amylodextrin.

Beyerinck, 1888 (*1*), described two types of nodules: One in which the bacteria gained the ascendancy and destroyed the interior, they themselves remaining alive; the other, in which the nodule i. e., the host plant, received the advantage, the bacteria being converted mostly into bacteroids, furnishing food for the plant but incapable of growth.

Nobbe and Hiltner, 1893 (*12*), inoculated peas with several pure cultures one of which gave particularly poor results, the plants being inferior in size and color to the others. The nodules produced by this culture were very numerous and large, but contained few bacteroids. This apparently was the condition which Hiltner in a later paper, 1904 (*8*), described as the fifth type of immunity, i. e., the organisms are so efficient in comparison with the plant that the latter is injured.

[1] Received for publication Sept. 14, 1927; issued January, 1928. Contribution from the Department of Agricultural Bacteriology, University of Wisconsin.

[2] Reference is made by number (italic) to "Literature cited," p. 1053.

In another paper Nobbe, Hiltner, and Schmid, 1895 (*13*), reported some work on the efficiency of cultures in cross inoculations. Organisms from *Robinia pseudacacia*, *Acacia lophanta*, *Vicia sepium*, and Pisum were used to inoculate Robinia, Acacia, and *Vicia villosa*. All four cultures produced nodules on both the Robinia and Acacia plants. The Robinia culture on Robinia and the Acacia culture on Acacia produced very large nodules; the other inoculations produced very small ones. The vetch did not form nodules when inoculated with either Robinia or Acacia cultures. Both the vetch and pea cultures formed nodules on vetch, the vetch inoculation producing large nodules on the secondary and tertiary roots, and the pea inoculation very much smaller nodules on the tertiary and quaternary roots. A summary of the dry weights and nitrogen percentages of this experiment is given in Table 1. From three such experiments in nitrogen-free sand, Nobbe, Hiltner, and Schmid (*13*) concluded that the various leguminous plants were benefited most readily if inoculated with bacteria from nodules of their own species.

TABLE 1.—*Plant weights and nitrogen percentages obtained by inoculating Robinia, Acacia, and Vicia Villosa with various cultures* [a]

Source of bacteria	Robinia		Acacia		Vicia villosa	
	Dry weight	Nitrogen in tops	Dry weight	Nitrogen in tops	Dry weight	Nitrogen in tops
	Gm.	*Per cent*	*Gm.*	*Per cent*	*Gm.*	*Per cent*
Robinia	7.4020	3.23	1.953	0.85	0.7829	1.46
Acacia lophanta	1.1582	1.77	6.943	1.44	.8658	1.59
Vicia sepium	.8584	1.45	1.248	1.05	9.1331	2.87
Pisum	1.4795	1.40	1.817	.92	1.0334	2.22

[a] From Nobbe, Hiltner, and Schmid

In analyzing the above data it must be remembered that this work was carried out before many of the later methods for the determination of pure cultures were devised or before cross-inoculation limitations were fully recognized. The differences obtained by the inoculation of vetch with the vetch and pea organisms, however, probably demonstrate a strain variation.

In 1900, Hiltner (*7*) reported some work indicating strain variations. Filtered Pisum nodule extract was applied to both Pisum and Lathyrus roots. The extract apparently contained some substance which caused a curling of the root hairs similar to that occurring with an invasion of the nodule organism. The curling of the Lathyrus however, was much slower, indicating that the organism in the pea nodule was more specific for peas (Pisum) than for sweet peas (Lathyrus).

Dehérain and Demoussy, 1900 (*4*), working with the white lupin concluded that the amount of nitrogen fixed by it depended on the types of nodules produced. They distinguished four types of nodules:

1. Small nodules placed on the main roots resembling a string of beads. The nitrogen-fixing ability of this form was high, the plants containing about 3 per cent nitrogen in the dry matter.

2. Medium-size smooth nodules, sometimes forming a collar about the top of the root. Plants with this type of nodule contained about 2 per cent nitrogen in the dry matter.

3. Half spherical nodules sometimes encasing the roots (vetch) or sometimes projecting from the roots (alfalfa). The plants having this form contained about 1 per cent nitrogen in the dry matter.

4. Enormously large nodules in the form of a raspberry. Plants of this type contained only 0.6–0.8 per cent nitrogen in the dry matter.

Moore, 1905 (*11*), made the following statement regarding the ability of the plant to receive help from certain types of nodules:

> Thus, if nodule-forming organisms be grown upon artificial media for a long time, where they are almost invariably in the rod condition, this form becomes so firmly established that plants inoculated with such cultures, although forming nodules, receive practically no benefit, the nodules remaining firm and hard and furnishing no nitrogen to the roots.

Harrison and Barlow, 1907 (*6*), discussing cross inoculation between pea and vetch, state:

> Cross inoculations have not been observed. Pea and vetch growing in the same flask, which was inoculated with vetch culture, formed abundant nodules and root infection occurred in the vetch, but the pea showed no infection whatever.

This is difficult to explain in the light of present knowledge, as pea and vetch are known to cross inoculate. There is a possibility, however, that Harrison and Barlow had a strain which inoculated the vetch only.

New York (Cornell) Experiment Station, 1918 (*10*), made a preliminary report on the efficiency of different strains of *Bacillus radicicola* in the production of nodules and the fixation of nitrogen when transferred from the true hosts to secondary ones. They stated that the organisms of peas transferred to vetch produced nodules, but without nitrogen fixation. No further report of this work has been found.

Bialosuknia, 1923 (*2*), from the results of his investigations drew the following conclusions in regard to host-plant specificity:

1. Bacteria of the pea in passing to broad bean form nodules but give a minimum effect.

2. This relationship is not changed by two plant passages.

Stevens, 1925 (*14*), working with various strains of the alfalfa root-nodule organism concluded:

> Strains of the alfalfa, sweet clover group of nodule bacteria differ under similar conditions in their ability to fix free nitrogen and to benefit the host plant.

He was able to show differences in the cultural, physiological, and serological characteristics of the various strains.

Wright, 1925 (*15*, *16*), demonstrated two types of soy-bean nodule organisms which varied greatly in their cultural, physiological, and serological properties. These types also differed widely in their ability to aid the plant in the fixation of nitrogen.

In addition to the observations reported in the literature differences were noticed in type of nodule development and plant growth in both greenhouse and field, due to the use of different strains of bacteria on peas. As a result of the above observations, experiments were carried out to study further the questions of strain variations and host-plant specificity.

MATERIAL AND METHODS

Seventeen strains of nodule bacteria were used; however, a major portion of the work was done with only a few of the strains which showed significant differences. These strains were all Wisconsin stock cultures of various ages, none of which had been through a plant passage since their addition to the Wisconsin culture collection. A summary of the histories of these strains is given in Table 2. It should be noted that strain 15 was secured by picking an isolated colony from a plate made of strain 8. Immediately before the experiments were begun, the cultures were tested twice for purity on milk and potato media, according to the method of Löhnis and Hansen 1921 (*9*). They were also tested three times for nodule formation and were all found to be efficient. During these preliminary tests differences in the type of nodule production were noticed.

TABLE 2.—*History of cultures used in experiments 1 to 5*

Wisconsin strain No.	Year secured	Source
2	1922	Isolated from a commercial pea culture.
3	1921	Isolated from pea nodule at Madison, Wis.
4	1921	Secured from University of Illinois, isolated from pea nodule.
5	1924	Isolated from pea nodule (Late Sweets), Urbana, Ill.
6	1918	Secured from U. S. Department of Agriculture, pea culture.
7	1918	Isolated from a commercial pea culture.
8	1916	Secured from U. S. Department of Agriculture, vetch culture.
9	1922	Isolated from commercial vetch culture.
10	1924	Isolated from pea nodule (Late Sweets), Urbana, Ill.
11	1924	Do.
13	1926	Secured from U. S. Department of Agriculture, Vicia faba culture.
14	1926	Secured from Kluyver (Holland) pea culture.
15	1924	Secured from University of Illinois; original source U. S. Department of Agriculture, vetch culture.[a]
20	1924	Isolated from pea nodule (Alaska pea), Urbana, Ill.
21	1924	Do.
22	1924	Do.
23	1924	Do.

[a] This culture was secured at the University of Illinois by replating the U. S. Department of Agriculture vetch culture numbered Wisconsin 8, above.

With the exception of the first experiment, the plants were grown on glacial sand. This sand was found to be very low in nitrogen, and as a result differences in nitrogen fixation were plainly evidenced in plant growth. The pots were covered with paper after being filled with sand and autoclaved at 15 pounds for 16 to 20 hours. The seed used in these experiments was freed of the nodule organism by immersion in hot water at 65° C. for four minutes. The plants were watered whenever necessary with sterile distilled water. An application of liquid culture solution was made every two weeks. The solution used was a modification of Bryan's, 1922 (*3*). The formula follows:

KCl	10. 0 gm.
$CaSO_4$	2. 5 gm.
$MgSO_4$	2. 5 gm.
$Ca_3(PO_4)_2$	2. 5 gm.
$FePO_4$	2. 5 gm.
K_2PO_4	2. 5 gm.

The salts were ground together in a mortar and used at the rate of 1½ gm. per liter of distilled water. After shaking, the solution was allowed to stand 24 hours. The clear solution was then decanted and sterilized.

EXPERIMENTAL DATA

EXPERIMENT 1

Growth of Green Admiral Peas in Nitrogen-Free Sand, Inoculated with Various Cultures

The white quartz sand used in this experiment was treated with hydrochloric acid until all nitrates had been leached out. It was then washed thoroughly with distilled water and made up for each pot as follows:

Nitrate-free sand	2, 500 gm.
Nitrate-free kaolin	10 gm.
Nitrate-free $CaCO_3$	1 gm.
Distilled water	275 c.c.

After sterilization, nine pots were planted with seeds inoculated with each strain. Six strains were used in this experiment. After two months' growth the plants were washed out of the pots for observation and analysis. The results are given in Table 3. The growing conditions during this experiment were very poor, the weather being too hot for the growth of peas. The results, however, are interesting since they show a wide variation in the nitrogen content of the plants inoculated with different cultures. Plants inoculated with strain 15 were especially low in nitrogen, suggesting that this strain fixes but little nitrogen.

Table 3.—*Dry weight and nitrogen content of Green Admiral peas inoculated with various cultures, and grown in the greenhouse in nitrogen-free sand; April, 1926*

Inoculated with strain No.—	Average weight per plant air dry	Nitrogen in water-free material	Total nitrogen per plant	Inoculated with strain No.—	Average weight per plant air dry	Nitrogen in water-free material	Total nitrogen per plant
	Gm.	*Per cent*	*Mgm.*		*Gm.*	*Per cent*	*Mgm.*
2	0.308	2.10	0.6468	15	0.333	1.88	0.6260
4	.330	2.36	.7788	20	.328	2.31	.7577
6	.376	2.01	.7558	Not inoculated	.336	1.80	.6048
10	.312	2.05	.6396				

EXPERIMENT 2

Growth of Green Admiral Peas in Sand, Inoculated with Various Cultures

The peas grown in glacial sand showed significant differences. (Table 4.) The uninoculated plants and those inoculated with strain 15 were of a yellowish-green color and very much shorter than the other plants. The plants inoculated with strain 13 also did not have a normal dark-green color, but were slightly tinted with yellow.

TABLE 4.—*Color, dry weight, and nodule formation of Green Admiral peas inoculated with various cultures and grown in the greenhouse in glacial sand; April, 1927*

Inoculated with strain No.—	Color of vines	Average weight per plant air dry	Type of nodule formation
		Gm.	
2	Normal green	0.990	Large nodules on taproot near surface of soil.
3	do	.915	Similar to No. 2.
4	do	.913	Large nodules on taproot near surface, also some scattered on lower roots.
5	do	.909	Similar to No. 4.
6	do	.993	Do.
7	do	1.044	Do.
8	do	.971	Similar to No. 2.
9	do	1.011	Do.
10	Pale green	.822	Similar to No. 4, but nodules somewhat smaller.
11	Normal green	.880	Similar to No. 4.
13	Pale green	.777	Nodules fairly well scattered over roots; many small ones.
14	Normal green	.911	Similar to No. 2.
15	Yellowish green	.400	Very small nodules scattered throughout roots.
20	Normal green	.955	Similar to No. 4.
21	do	.811	Do.
22	do	.840	Do.
23	do	.795	Do.
Not inoculated	Yellowish green	.314	An occasional nodule; located anywhere on roots.

It is interesting to note that a relationship seems to exist between the position of the nodules on the roots and the benefit derived by the plant. The cultures which were of greatest benefit to the plant produced large nodules, practically all of which were located on or near the primary root and near the surface of the soil. Strain 15 produced a large number of nodules, all of which were exceedingly small. These were scattered over the entire root system, as many appearing on the lower as on the upper roots. Strain 13, and to a lesser extent strain 10, produced medium-size nodules which were scattered over the roots.

The uninoculated plants and the strain 15 inoculated plants produced only a few pods which contained 2 or 3 peas per pod. All other plants produced several large pods containing 5 and 6 peas per pod.

EXPERIMENTS 3 AND 4

GROWTH OF ALASKA PEAS IN SAND, INOCULATED WITH VARIOUS CULTURES

The plants in experiment 3 were harvested and the observations recorded (Table 5) after six weeks' growth. The differences in growth are shown in Figure 1. The pots are numbered with the number of the strain used in inoculating them. The plants which showed a pronounced yellowish color are the uninoculated, No. 12 and No. 15. Plants inoculated with strain 13 did not grow as well as the others, but had a healthy green color. Pot 12 was inoculated with a *Bacillus radiobacter* culture and the plants produced no nodules. The difference in color of plants inoculated with strain 15 and those inoculated with the other strains is well defined. Although numerous nodules were produced on the roots of these plants, there was apparently no benefit received as the plants did not develop any further than the controls.

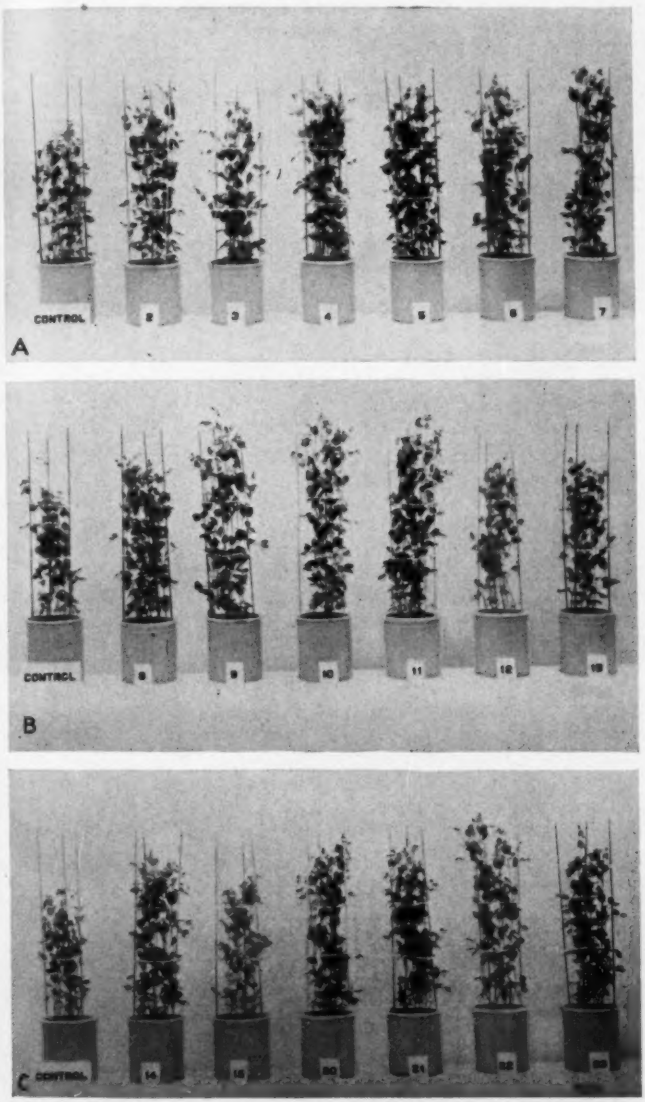


FIG. 1.—Variations in the growth of Alaska peas (*Pisum sativum*) induced by inoculation with various strains of bacteria: A, Pots containing plants uninoculated and inoculated with strains 2, 3, 4, 5, 6, and 7. B, Pots containing plants uninoculated and inoculated with strains 8, 9, 10, 11, 12, and 13. C, Pots containing plants uninoculated and inoculated with strains 14, 15, 20, 21, 22, and 23

TABLE 5.—*Color, height, dry weight, and nodule formation of Alaska peas inoculated with various cultures and grown in the greenhouse in glacial sand; February, 1927*

Inoculated with strain No.—	Color of vines	Height of plant	Average weight per plant air dry	Type of nodule formation
		Inches	*Gm.*	
2	Normal green	18	0.754	Large nodules on taproot near surface of soil.
3	do	18	.588	Similar to No. 2.
4	do	19	.673	Many nodules on taproot and scattered on upper roots.
5	do	20	.683	Similar to No. 4.
6	do	21	.664	Do.
7	do	20	.759	Do.
8	do	19	.636	Similar to No. 2.
9	do	20	.690	Do.
10	do	20	.646	Nodules somewhat smaller and more scattered than No. 4.
11	do	19	.575	Similar to No. 4.
13	do	17	.606	Nodules somewhat smaller and more scattered than No. 4.
14	do	21	.572	Similar to No. 4.
15	Yellowish green	14	.535	Many very small nodules scattered all over roots.
20	Normal green	21	.553	Similar to No. 4.
21	do	20	.541	Do.
22	do	20	.570	Do.
23	do	22	.562	Do.
Not inoculated	Yellowish green	13	.539	One pot o fsix contained a few nodules.

The nodules on the plants inoculated with strain 15 differed greatly from those on the other plants. The nodules were very small and scattered very low on the roots. (Fig. 2, A.) Practically no nodules formed on the taproot. Most of the nodules appeared undeveloped. The roots inoculated with strain 3 had large nodules on the taproot and upper side roots. (Fig. 2, B.) Practically no nodules appeared low in the roots. The nodules produced by all strains except 10, 13, and 15 were very similar to this type. Strains 10 and 13 gave nodules which seemed to be intermediate in type between Nos. 3 and 15.

Experiment 4 was a repetition of Experiment 3, and the results were very similar. (Table 6.) All of the cultures except strain 15 showed increases in growth over the controls. The plants inoculated with strain 15 produced even less dry weight than the controls. This, however, may have been due to the fact that the controls formed a few nodules, apparently from air contamination.

TABLE 6.—*Color, dry weight, and nodule formation of Alaska peas inoculated with various cultures and grown in the greenhouse in glacial sand; February, 1927. Duplicate of experiment shown in Table 5*

Inoculated with strain No.—	Color of vines	Average weight per plant air dry	Type of nodule formation
		Gm.	
2	Normal green	1.073	Large nodules practically all on taproot.
3	do	1.110	Similar to No. 2.
4	do	1.128	Nodules on taproot and scattered in upper roots.
5	do	1.053	Similar to No. 4.
6	do	1.063	Do.
7	do	1.119	Do.
8	do	1.146	Do.
9	do	1.052	Similar to No. 2.
10	do	1.044	Nodules somewhat smaller and more scattered than No. 4.
11	do	1.054	Similar to No. 2.
13	Pale green	.922	Similar to No. 10.
14	Normal green	.932	Similar to No. 2.
15	Yellowish green	.647	Very small nodules scattered throughout root system.
20	Normal green	.920	Similar to No. 4.
21	do	.927	Do.
22	do	.950	Do.
23	do	.929	Do.
Not inoculated	Yellowish green	.729	An occasional nodule.

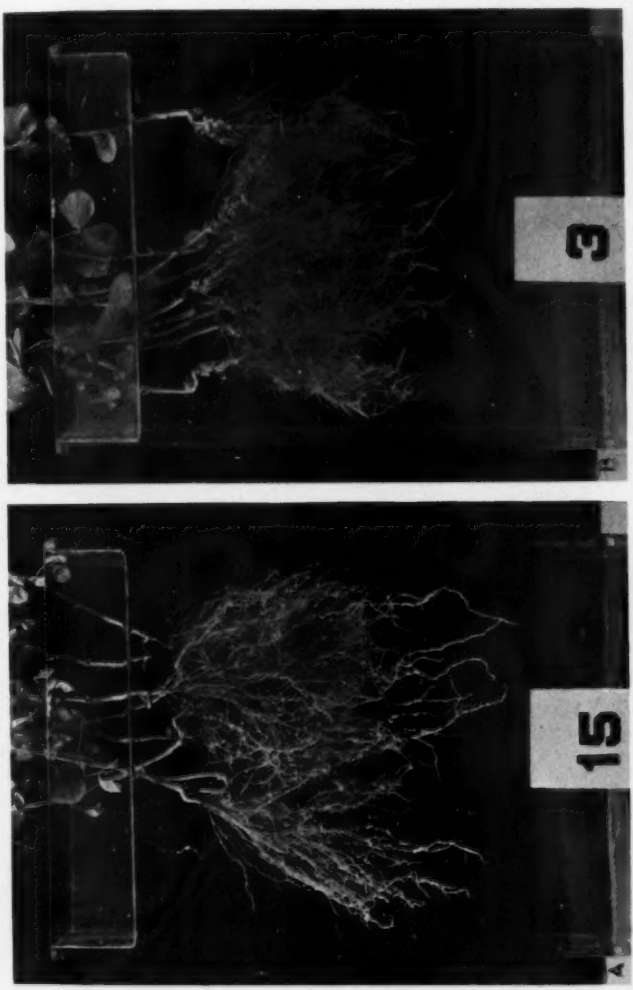


FIG. 2.—Roots of Alaska peas (*Pisum sativum*) showing type of nodule formation: A, Inoculated with strain 15. Numerous small nodules, scattered all over the root system. B, Inoculated with strain 3. Relatively few large nodules, placed almost exclusively on the upper parts of the root system

EXPERIMENT 5

GROWTH OF VICIA FABA, LATHYRUS ODORATUS, VICIA VILLOSA AND LENS ESCULENTA IN SAND, INOCULATED WITH VARIOUS CULTURES

The fact that strain 13, which was known to have been isolated from *Vicia faba*, did not give as good results on peas as the pea cultures brought up the question of whether it would benefit its own original host plant more than would a pea culture.

VICIA FABA

Vicia faba was, therefore, inoculated with strains 13 and 20. The plants inoculated with strain 20 (Table 7) were somewhat better than the controls, but similar to them, both having wilted and dried lower leaves. (Fig. 3, A.) The *Vicia faba* culture, strain 13, produced a very good growth of plants, the dry matter produced being greater than that of the plants inoculated with strain 20.

The nodules produced by strain 20 (fig. 4, A) were very small, and similar to those of the lupin type. They were fairly well scattered over the root system. The nodules produced by strain 13 (fig. 4, B) were very large and well formed, being placed on the upper roots near the surface of the soil. The roots of a plant from an uninoculated pot (fig. 4, C) contained no nodules.

TABLE 7.—*Color, dry weight, and nodule formation of broad bean inoculated with strains 13 and 20 and grown in the greenhouse in glacial sand; February, 1927*

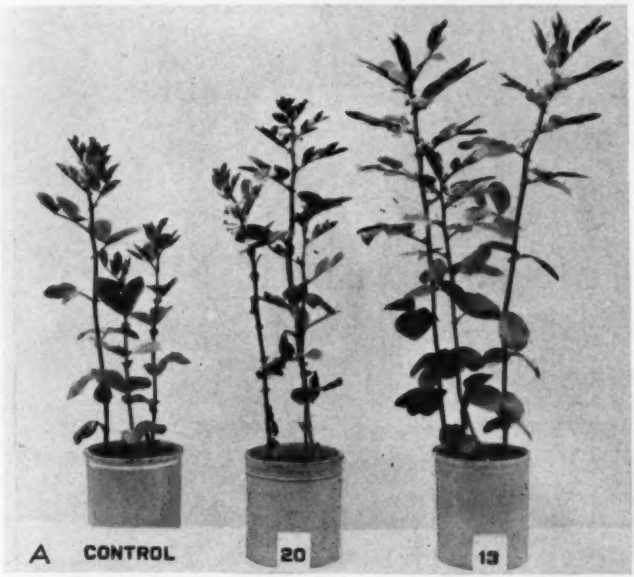
Inoculated with strain No. —	Color of vines	Average weight per plant air dry	Type of nodule formation
		Gm.	
13	Normal green; all leaves healthy	0.077	Many large, well-formed nodules.
20	Pale green; lower leaves dried	.066	Many small, scattered, underdeveloped nodules.
Not inoculated	Yellowish green; lower leaves dried.	.049	No nodules.

LATHYRUS ODORATUS

On sweet peas as on garden peas strain 15 gave very inferior results. (Table 8.) It appeared that the plants were not only suffering from a nitrogen deficiency, but that the organisms on the roots were actually parasitic. The dry weight of the plant inoculated with strain 15 was even less than the weight of the uninoculated plants.

TABLE 8.—*Color, height, dry weight, and nodule formation of sweet peas inoculated with various cultures and grown in the greenhouse in glacial sand; April, 1927*

Inoculated with strain No.—	Color of vines	Height of plants	Average weight per plant air dry	Type of nodule formation
		Inches	*Gm.*	
3	Normal green	20-26	1.363	Large well-formed nodules on taproot near surface of soil.
13	Pale green	16-18	.488	Fair-sized nodules on upper roots; some scattered low in roots.
15	Yellowish green	4-8	.310	Very small nodules scattered throughout root system.
20	Normal green	20-30	1.268	Large well-formed nodules on taproot near surface of soil.
Not inoculated	Yellowish green	6	.429	No nodules.



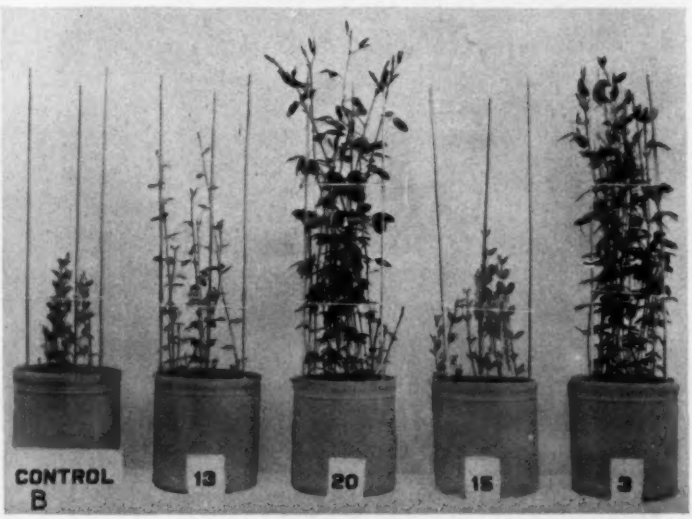


FIG. 3.—Variation in plant growth induced by inoculation with various strains of bacteria: A, Broad bean (*Vicia faba*). Plants inoculated with strain 13 have made better growth than those inoculated with strain 20. B, Sweet pea (*Lathyrus odoratus*). Plants inoculated with strain 20 have made much better growth than those inoculated with strain 13

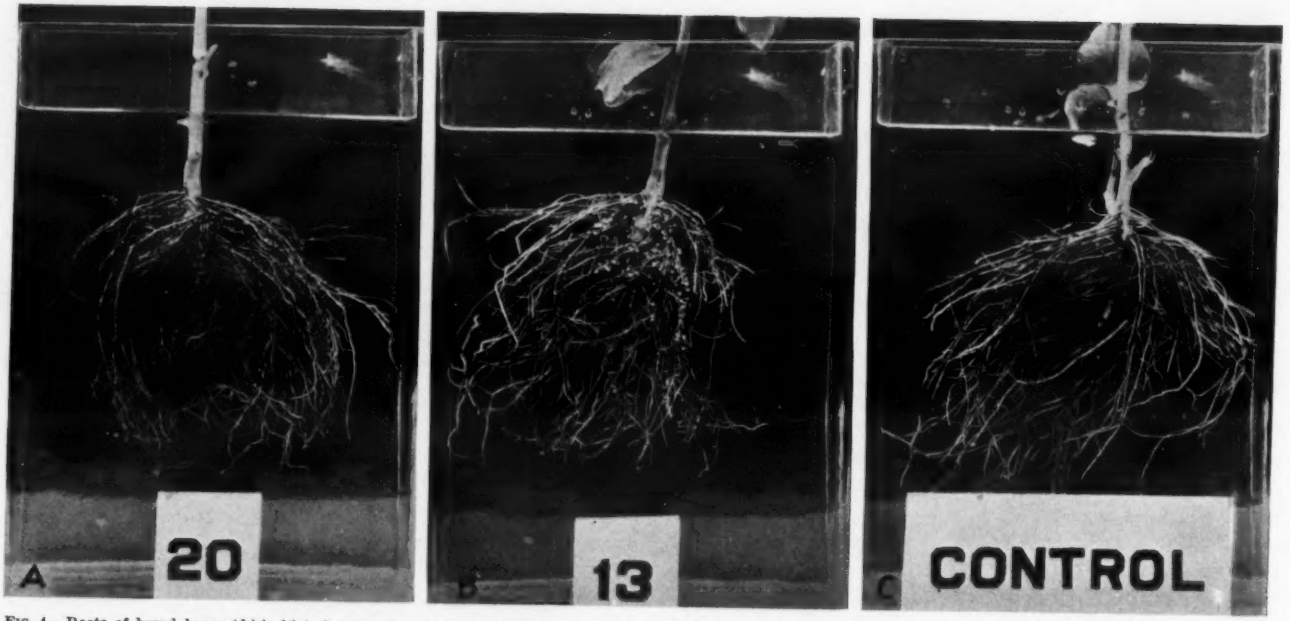


FIG. 4.—Roots of broad beans (*Vicia faba*) showing type of nodule formation: A, Inoculated with strain 20. Numerous small nodules, scattered over the entire root system. B, Inoculated with strain 13. Relatively few large nodules, placed on the upper parts of the root system. C, Uninoculated. No nodules

The plant growth of the sweet peas was much influenced by the use of different cultures. (Fig. 3, B.) The plants inoculated with strain 15 were yellowish light green similar to the controls. Plants inoculated with strain 13, although producing more dry matter, were very thin and weak. Strains 3 and 20 gave excellent dark green plants.

The nodules on the plants inoculated with strain 13 (fig. 6, A) were of medium size and scattered fairly well on the side roots near the top of the plant. The roots inoculated with strain 15 (fig. 6, B) had exceedingly small nodules scattered throughout the root system, many being located low on the roots. The nodules produced by strain 20 (fig. 6, C) were large and located on the taproot and upper side roots near the taproot.

VICIA VILLOSA

Differences produced in the growth of hairy vetch inoculated with various cultures are given in Table 9. Strain 13 apparently produced just as good a growth of vetch, as did either strains 3 or 20. (Fig. 5, A.) The nodule placings on plants inoculated with strains 3, 13, and 20 were very similar. Strain 15, however, produced much smaller nodules somewhat lower on the roots.

TABLE 9.—*Color, dry weight, and nodule formation of hairy vetch inoculated with various cultures and grown in the greenhouse in glacial sand; April, 1927*

Inoculated with strain No.—	Color of vines	Average weight per plant air dry	Type of nodule formation
		Gm.	
3	Normal green	0.375	Large well-formed nodules scattered on roots.
13	do	.395	Do.
15	Yellowish green	.150	Very many small nodules scattered on roots.
20	Normal green	.343	Large well-formed nodules scattered on roots.
Not inoculated	Yellowish green	.163	None.

LENS ESCULENTA

The results obtained in the growth of the lentil are given in Table 10. Figure 5, B, shows the relative differences in growth. The results were very similar to those obtained with the vetch, strain 15 being the only one which did not show a great benefit to the plant.

TABLE 10.—*Color, dry weight, and nodule formation of lentil inoculated with various cultures and grown in the greenhouse in glacial sand; April, 1927*

Inoculated with strain No.—	Color of vines	Average weight per plant air dry	Type of nodule formation
		Gm.	
3	Normal Green	.735	Many large nodules in upper roots.
13	do	.626	Many fairly large nodules in upper roots.
15	Yellowish green	.450	Many very small nodules scattered throughout roots.
20	Normal green	.063	Many large nodules on tap and upper roots.
Not inoculated	Yellowish green	.455	An occasional nodule.

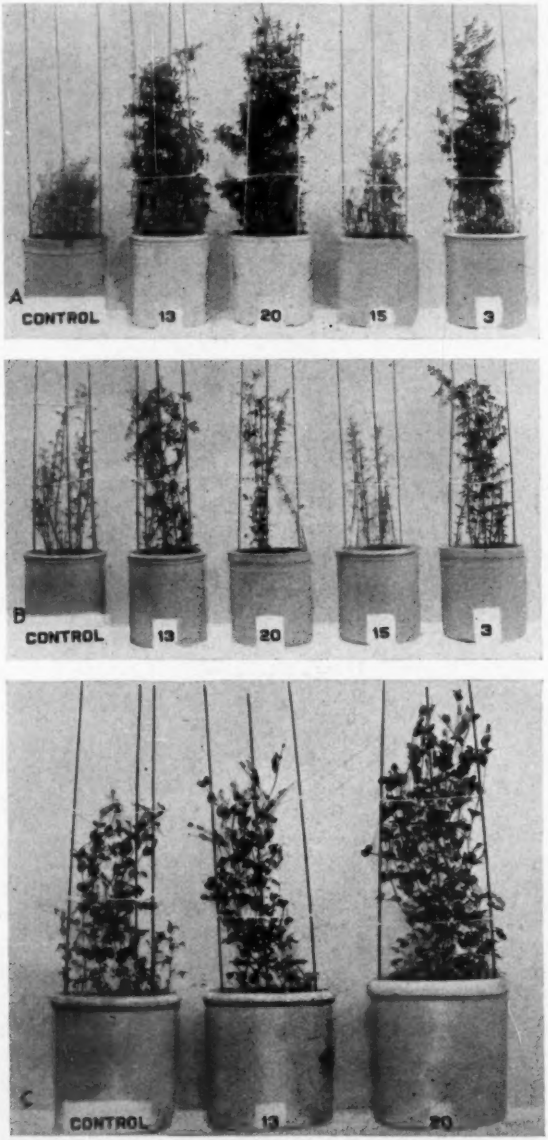


FIG. 5.—Variation in plant growth induced by inoculation with various strains of bacteria: A, Hairy vetch (*Vicia villosa*). Plants inoculated with strains 13 and 20 have made about equal growth. B, Lentil (*Lens esculenta*). Plants inoculated with strains 13 and 20 have made about equal growth. C, Green Admiral Peas (*Pisum sativum*). Plants inoculated with strain 20 have made better growth than those inoculated with strain 13

DISCUSSION

The fact that strain variations may exist which affect the ultimate benefit derived by the plant is clearly demonstrated. A nodule organism which originally was isolated from *Vicia faba* (strain 13) gave excellent plant growth when used with *V. faba* in comparison with a culture (strain 20) originally isolated from pea. (Fig. 3, A.) This relationship was exactly reversed when the two cultures were used to inoculate peas, the pea strain (No. 20) giving the better results. (Fig. 5, C.) On vetch and on lentil the *V. faba* strain apparently was as efficient as any of the pea strains. On sweet peas, however, a great difference was again shown, the inoculation with the pea cultures giving very good results in comparison with the *V. faba* culture.

The location of the nodules in a root system and the size of the nodules seem to give an indication of the effectiveness of a culture. Strain 13 on *Vicia faba* (fig. 4, B) produced abundant, large nodules in the upper roots. This culture on sweet peas (fig. 6, A) produced smaller nodules scattered over the root system. With strain 20 the reverse took place. The nodules on the sweet pea were located on the taproot and other upper roots (fig. 6, C), while those on the *V. faba* were scattered throughout the root system (fig. 4, A). In general, throughout this work the cultures which were beneficial to their host plants produced large nodules on the taproot or other upper roots of the plant. Those cultures which were not so beneficial formed small nodules scattered throughout the root system.

In each of the experiments on all of the host species strain 15 was shown to be unable to aid the plant. Although an abundance of nodules was always formed, the plants never grew any better than the uninoculated plants. In fact, the growth of the plants inoculated with strain 15 was often poorer than that of plants having no nodules. The possibility exists that this organism, though able to stimulate the plant in the formation of nodules, is yet unable to fix nitrogen, and that any nitrogen going into the make-up of the organism is derived from the plant.

CONCLUSIONS

Not all strains of the pea-nodule organism are identical in their ability to aid the host plant. One strain was found which produced nodules on all five of the host species studied, but which did not benefit the plant.

A strain specificity has also been demonstrated. A culture originally isolated from broad bean (*Vicia faba*) produced better results on *V. faba* than did a culture originally isolated from peas. The pea culture, on the other hand, gave much better results on both peas and sweet peas than did the *V. faba* culture.

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FIG. 6.—Roots of sweet peas (*Lathyrus odoratus*) showing type of nodule formation: A, Inoculated with strain 13. Several medium-sized nodules, scattered over the entire root system. B, Inoculated with strain 15. Numerous small nodules, scattered over the entire root system. C, Inoculated with strain 20. Relatively few large nodules, placed on the upper parts of the root system

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